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Thesis
Erakıncı G. Searching for antibodies against parasites in donors. İzmir: Ege University Health Sciences Institute. 1997.
Article in Electronic Format
Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis (serial online) l995 Jan-Mar (cited 1996 June 5): 1(1): (24 screens). Available from: URL: http:/ www.cdc.gov/ncidodlElD/cid.htm.

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English title, author names and institutions.
Abstract (average 200-400 word)
Introduction
Methods
Results
Discussion and conclusion
References (most 40)
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MIDDLE BLACK SEA JOURNAL OF HEALTH SCIENCE

DECEMBER 2019 **VOLUME 5** **ISSUE 3**

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EDITORIAL

As we complete our fifth year,

Despite all the troubles and difficulties of our publication lives, the excitement and happiness we live in is worth everything. We will reach our goals by running together with you by growing and getting stronger in future five years…

We are proud to pass from difficulties in finding articles for our first issue to the level of article selection now. Our plans to continue publishing in different academic areas in the future are ready…We walk confidently together with the academic world.

We would like to thank the editorial board, referees and authors for their contributions in our issues that have been published so far and wish them peace in the new year.

PhD, Assoc. Prof. Ülkü KARAMAN

Editor

Middle Black Sea Journal of Health Science December 2019; 5(3):162-167

RESEARCH ARTICLE

BRAF Expression in Keratoacanthoma

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Abstract

Objective: Mutations in genes encoding proteins along the RAS-RAF-MEK-ERK pathway have been detected in a variety of tumor entities, including malignant melanoma, thyroid, colon, over carcinomas and some sarcomas. The increased activity of BRAF V600E leads to downward signalization activation via mitogen-activated protein kinase (MAPK), which plays an important role as cell growth, differentiation and survival regulator. Latest data show BRAF undergoes mutation in nearly 7% of cancers and this situation makes BRAF another important oncogene in this pathway. We aimed to evaluate the relationship between keratacanthoma and BRAF expression.

Methods: 28 cases of keratocanthomas were included in this study. Sections were taken from the selected blocks with a thickness of 3 microns with poly-lysine coating. BRAF antibody was applied to the tissues. The obtained preparations were evaluated by light microscopy. It was rated according to the degree of staining in epidermis.

Results: Areas showing cytoplasmic staining with BRAF were evaluated in sections. It was observed that there was no staining in the keratocanthomas, and staining in sebaceous glands and sweat glands in peripheral basal cells. It was also noted that the sweat glands had more stain than the sebaceous glands. The cases included 18 males and 10 females with ages varying from 33 to 85 years. The duration of the lesions was between one month and one year. Lesion dimensions varied from 5 to 70 mm, with mean size of 21 mm. There were 14 cases (50%) with head and neck localization, and 14 cases (50%) with localization other than the head and neck.

Conclusion: As a result, it has been concluded that BRAF mutation may not be involved in keratoacanthoma.
Key words: BRAF; keratoacanthoma; expression.

Suggested Citation: Onder S, Erdem H, Akcay Celik M. BRAF expression in keratoacanthoma. Middle Black Sea Journal of Health Science, 2019; 5(3): 162-167

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Introduction

The basis of the mitogen-activated protein kinase (MAPK) pathway is a signal pathway involving RAS, RAF, mitogen and extracellular-regulated protein kinase kinase (MEK) and extracellular signal regulating kinase (ERK) and is active in many tumors due to mutations in the RAS or RAF families. Discovery of mutations in one of the RAF kinase family members of BRAF (v-raf murine sarcoma viral oncogene homolog B) contributed greatly to advances in melanoma research and it is generally associated with early-stage melanoma (Abildgaard et al., 2015).

Additionally, BRAF mutations are observed with high frequency in melanocytic nevi (Davies et al., 2002; Pollock et al., 2003; Kumar et al., 2004). This clearly shows the role of BRAF in early melanomagenesis (Tsao et al., 2012). Latest data show BRAF undergoes mutation in nearly 7% of cancers and this situation makes BRAF another important oncogene in this pathway (Garnett et al., 2004).

The highest incidence of BRAF mutations is in malignant melanoma (27-79%), papillary thyroid cancer (36-53%), colorectal cancer (5-22%) and serous ovarian cancer (30%). However, low incidence mutations (1-3%) may occur in a variety of other cancers (Davies et al., 2002; Garnett et al., 2004).

Keratoacanthoma (KA) is a dome-shaped lesion filled with keratin originating in hair follicles. It is commonly observed in humans and the cause of the tumor is not fully known. However, currently the histopathologic diagnosis criteria, prognosis and treatment protocols are still controversial. Names used for KA include molluscum sebaceum, pseudotumor, regressing tumor and self-healing squamous cell carcinoma (SCC) (Kwiek et al., 2016).

Histopathologically KA has some defined characteristics (Clausen et al., 2006). The surface epithelium at the lateral margin of the tumor appears normal; however, there is a clear angle observed between the lesion and the upper epithelium at the lip section of the crater in the center of the lesion. The crater is filled with keratin and the epithelial cells at the base of the lesion proliferate toward the bottom and generally induce a significant chronic inflammatory response. Dyskeratosis may cause confusion with well-differentiated SCC (Chauhan et al., 2011). The architecture of the tumor is more important than cytologic features for diagnostic procedures (Chauhan et al., 2011) because it may show microscopic features of SCC such as lesion infiltration and cellular atypia (Neville et al., 2009). The most common concern remains the boundary between malignancy and benignity of the tumor. This indecision is difficult for both clinicians and researchers but may be key to understanding the regression of this tumor (Kwiek et al., 2016).

In this study we aimed to investigate the BRAF expression in KA cases.

Methods

Twenty-eight cases of keratoacanthoma were included in this study. Hematoxylin-eosin slides of tissue samples were re-examined, and appropriate paraffin blocks were selected. Sections were taken from the selected blocks with poly-lysine slides 3 microns thick. BRAF antibody was applied to the tissues. Immunohistochemical staining was performed by deparaffinization, dehydration and incubation in buffered citrate (1/200 dilution). Staining was performed using Ultra Vision Polyvalent, HRP-AEC kit (Neomarkers-Biogen, Lab Vision Corp. USA). The obtained slides were evaluated using light microscopy. They were graded according to the degree of staining in the epidermis (Figure 1-3). Cytoplasmic positive areas with BRAF were evaluated in the sections (Adackapara et al., 2013).

Descriptive analysis was used for statistical analysis in this study.

The trial was performed in accordance with clinical practice and the Helsinki Declaration. Approval was granted by the local ethics committee of Ordu University (2018-142).

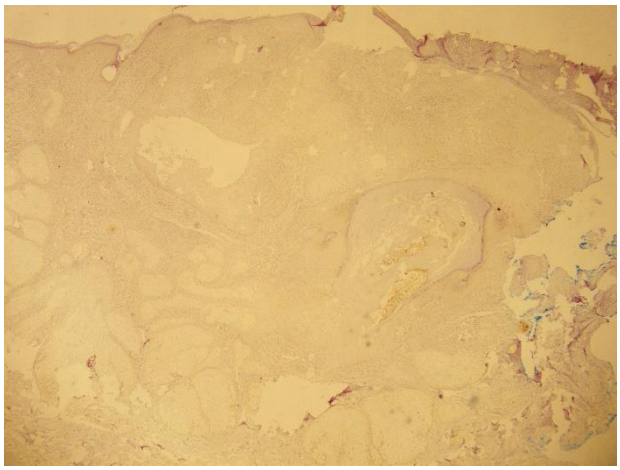

Figure 1. Loss of BRAF expression in crater cells (x40).

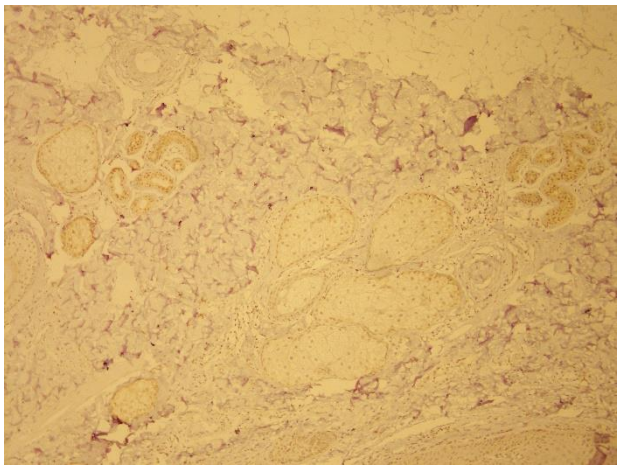

Figure 2. BRAF expression in sweat glands and sebaceous glands (x100).

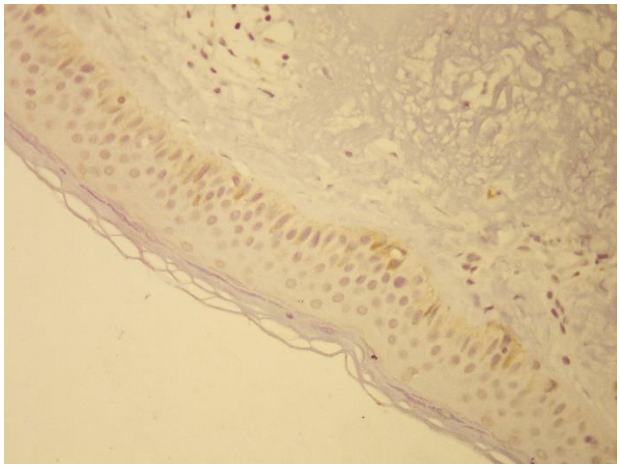

Figure 3. BRAF expression in basal cells outside crater (x400).

Results

A total of 28 cases with KA were evaluated. Positive areas with BRAF were evaluated in the sections. It was observed that there was no staining in the keratinocyte, and there was staining in sebaceous glands and sweat glands and peripheral basal cells outside the crater. It was also noted that the sweat glands had more stain than the sebaceous glands. The cases included 18 males and 10 females with ages varying from 33 to 85 years (mean 50). The duration of the lesions was between one month and one year. Lesion dimensions varied from 5 to 70 mm, with mean size of 21 mm. There were 14 cases (50%) with head and neck localization, and 14 cases (50%) with localization other than the head and neck.

Discussion

KA is a benign keratinocytic tumor sourced in hair follicles (Neville et al., 2009). It is encountered as clinically sporadic, solitary lesion showing rapid growth over four to five weeks and then resolving after six months (Aksoy B et al., 2017). Numerous studies have investigated the differences between KA and SCC in terms of cellular proliferation, apoptosis and cell cycle, and histomorphological regulation of molecules (Tan et al., 2013). In the literature there are contradictory ideas related to the nature of KA. Just as KAs are accepted as a subgroup of SCC or a precancerous SCC lesion, some authors accept them as benign lesions completely different from SCC (Aksoy et al., 2017). Though KAs mostly regress spontaneously, some immunosuppressive patients with KA were reported to develop metastasis. This led to debate about whether KA is a different entity or a variant of cutaneous SCC (Putti et al., 2004). Unfortunately, there are no clinical signs to differentiate KA from SCC with high specificity and sensitivity (Aksoy et al., 2017).

Among etiologic factors causing KAs are immunosuppression, ultraviolet radiation, chemical factors, genetic factors, BRAF inhibitors (vemurafenib, dabrafenib), hedgehog pathway inhibitors (vismodegib) and foreign material like tattoos or fillers (Aksoy et al., 2017; Tan et al., 2013; Putti et al., 2004; Hodak et al., 1993). Since the beginning of use of BRAF inhibitor treatment for melanoma and hedgehog pathway inhibitor treatment for advanced basal cell carcinoma, the incidence of KA has increased (Bell et al., 2015; Sarah et al., 2015). BRAF inhibitors are shown to lengthen survival in stage 4 metastatic melanoma patients with BRAF V600E mutation. However, development of KA, verrucous keratosis and cutaneous SCC is a disadvantage of the medication (Anforth et al., 2012).

Recent evidence in vitro and in vivo suggests that selective BRAF inhibitors can induce activation of downstream elements of the MAPK pathway in BRAF wild-type cancer cells (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010). A recent study showed BRAF inhibitors affect genetically abnormal keratinocytes causing formation of de novo lesions (Alloo et al., 2012).

In a study, data indicates that RAS mutations are present in approximately 60% of cases treated with BRAF inhibitors. The researchers reported mutations present may cause tendency for SCC and KA development (Fei et al., 2012). In this study,

absence of BRAF supports the presence of RAS mutation in KA.

There are many studies examining the relationship of squamoproliferative lesions due to BRAF inhibitors such as KA, verrucous papilloma (VP), SCC. In a study the authors evaluated both the MAPK pathway and the presence of (Human Papilloma Viruses) HPV and other polyomaviruses (HPyV), especially Merkel cell polyomavirus (MCPyV), in a group of BRAF inhibitor induced verrucous papillomas (VP), keratoacanthomas and squamous cell carcinomas. They detected HPV only in 2 VPs and they didn't show any relation between KAs and HPV (Frouin et al., 2014). In an another study, Schrama et al detected HPV in 14 cSCC, 3 KA and one acanthoma samples and all were HPV positive (Schrama et al., 2014). The results are controversial as to whether or not HPV has an effect on KA development in patients under melanoma treatment. In an another molecular study authors compare the mutational profiles of lesions after treatment with a BRAF inhibitor, with similar lesions arising sporadically. HRAS mutations were common among the BRAF inhibitor-induced lesions. So, despite similar histomorphological appearances, the underlying molecular mechanisms may be different. In addition, within the BRAF inhibitor-associated group, the lesions designated as KAs and BRAF inhibitor-associated verrucous keratoses had a similar mutational profile (mutations in PIK3CA, APC, and HRAS), which was distinct from squamous cell carcinomas (FGFR3, CDKN2A, and STK11) (Clynick et al., 2015).

The mechanism underlying SCC development in patients treated with RAF inhibitors is currently being actively researched (Hatzivassiliou et al., 2010; Heidorn et al., 2010; Poulikakos et al., 2010). A study by Arnault et al. (Arnault et al., 2012) compared normal skin biopsy samples taken from patients treated with sorafenib with skin with normal appearance taken from patients treated with placebo. They showed the histologic sections of patients treated with sorafenib had increased rates of Ki67 and phosphorylated ERK staining in keratinocytes. They suggested the MAPK signaling was actually increased in patients treated with sorafenib and this probably caused increased keratinocyte proliferation. However, paradoxical activation of MAPK signaling alone may not be sufficient to induce SCC and KA. With this aim, Oberholzer et al. (Oberholzer et al., 2012) compared patients treated with vemurafenib (30%) and sorafenib (11%) with a control group (3.2%) and identified RAS activated mutations were more common in SCC and KA of treated patients. Previously existing RAS mutations in keratinocytes (probably due to sun exposure or viral infections) may be a "second hit" on the paradoxical activation pathway beginning with MAPK signaling by the RAF inhibitor, and this will be sufficient for tumor development.

In our study, expression of BRAF was not seen in the KA crater cells. Expression was seen in basal cells outside the crater of the lesions. In addition, expression of BRAF was seen in the sweat and sebaceous glands. We noted that the expression in the sweat gland was greater than in the sebaceous glands.

In a study of melanomas with anorectal location, positive BRAF expression was observed in the control group and anorectal glands. However, BRAF expression was negative in all cases of primary anorectal melanoma (Tse et al., 2016). Other published studies found that among 323 colorectal carcinoma cases with wild-type BRAF by sequence analysis, 196 cases (61%) did not stain with the BRAF antibody (Estrella et al., 2015).

Conclusion

In this study, expression in the basal cell and gland is nonspecific. No significant staining was observed in the cases. Extensive research is needed to demonstrate the relationship between BRAF and KA. The staining of the glands is thought to be nonspecific because it was not associated with histopathology of keratoacanthoma.

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RESEARCH ARTICLE

The Factors Affecting Smoking Cessation and Efficacy of Quitting Methods

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Abstract

Objective: We aimed to compare the demographic-sociocultural characteristics of quitting and non-quitting individuals and to evaluate the factors affecting smoking cessation and success rates of treatment methods.

Methods: 179 patients were included in the study. Demographic characteristics, smoking behaviour, Fagerström nicotine dependence level were recorded. The treatment results at the end of the first year were evaluated. The efficacy of treatment methods used in the treatment of smoking dependence, gender, occupation, education, comorbidity, and family history were recorded in quitting and non-quitting individuals. Statistical analysis was performed using Statistical Package for Social Sciences Version 21.0 (SPSS-21.0).

Results: Of the 179 patients evaluated, 118 (65.6%) were male and 71 (39.7%) were married. The mean age was 41.5 ± 13.7 years. At the end of 12 months follow-up, 57 (31.8%) had been quitted smoking. There was no difference between the quit and non-quit groups in terms of gender, age, working status and education level. It was found that being married and having no additional disease significantly increased smoking cessation rate ($p = 0.049$, $p = 0.010$, respectively). In both groups, combination of behavioural treatment and varenicline was the most common method. There was no significant difference among the pharmacological methods used.

Conclusion: In our study, smoking cessation rate was found to be 31.8%. The efficacy of different treatment modalities was similar. Despite the current treatments, the rate of smoking cessation is not at the desired level and further studies are needed to develop new methods for smoking cessation treatment.

Key words: Tobacco use, smoking reduction, smoking cessation

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Introduction

One of the biggest social problems in the world and in our country is cigarette addiction (Karnath 2002). Cigarette addiction is defined by the World Health Organization (WHO) as a chronic disease with recurrences (WHO, Report on the Global Tobacco Epidemic, 2019). Active and passive smoking affects all organs, tissues and systems, leading to death, disease and disability (Mackay and Eriksen, 2002; Fried, 2003).

In Global Adult Tobacco Survey, 19.2 million people in Turkey (31.6%) currently use tobacco products. The prevalence of tobacco use is still higher in men (44.1%) than in women (19.2%) (Global Adult Tobacco Survey, Turkey 2016).

In the world, more than a billion smokers are addicts. Most of these addicts would like to quit, but 5% can receive comprehensive services for the treatment against tobacco addiction. Countries should establish effective and inexpensive intervention programs for those who smoke and willing to quit. The fight against smoking is the duty of all physicians and it is recommended to support the quitting efforts of individuals using tobacco (Mackay and Eriksen, 2002).

It is known that the programs implemented in specialized smoking outpatient clinics increase the success of smoking cessation (Parrott and Godfrey, 1998).

The aim of this study was to investigate the demographic-sociocultural characteristics of patients followed up at the smoking cessation clinic, factors affecting smoking cessation, and the success rates of smoking cessation methods.

Methods

The files of the patients who applied to the smoking cessation clinic of Ufuk University Dr. Rıdvan Ege Hospital between the dates of January 2010 and December 2012 were retrospectively reviewed. The criteria for inclusion in the study were being> 18 years of age and follow-up in our smoking cessation clinic for at least one year. Patients who were followed-up for less than one year, ≤18 years of age and who had missing data on their file, and missing information about the smoking cessation treatment response were excluded from the study. All patients had Fagerström nicotine dependence test at their admission. Determination of addiction level is important for the choice of treatment and success of cessation. The Fagerström Test for Nicotine Dependence (FTND) is the most commonly used test for the assessment of cigarette dependence in daily practice (Demir, 2008). The Fagerström Tolerance Test was first proposed in 1978, and in 1991, Heatherton et al. (1991) called Fagerström Tolerance Scala. Adapting the test to Turkish was made by Uysal et al. (2004). Demographic characteristics, smoking behaviour and attitudes of the patients, FTND score, comorbid medical or psychiatric diseases and drugs used were recorded.

Behavioural therapies consisting of practical applications were recommended in our smoking cessation clinic. In order to cope with early withdrawal symptoms and late effects, the patient was advised to stay away from smoking environments, to be active, to use chewing gum, to perform deep breathing exercises and to prevent weight gain. Patients were given a telephone number to call if they needed it. They were evaluated psychologically during their regular follow-up. The treatment method (pharmacological and/or behavioural treatment), duration of treatment, gender, occupation, education, comorbidity, family history, smoking at home and addiction scores were recorded in subjects who quit and could not quit. The results of the treatment at the end of the first year were evaluated by using file data and contacting the patients by telephone.

Design of the study was summarized in figure 1.

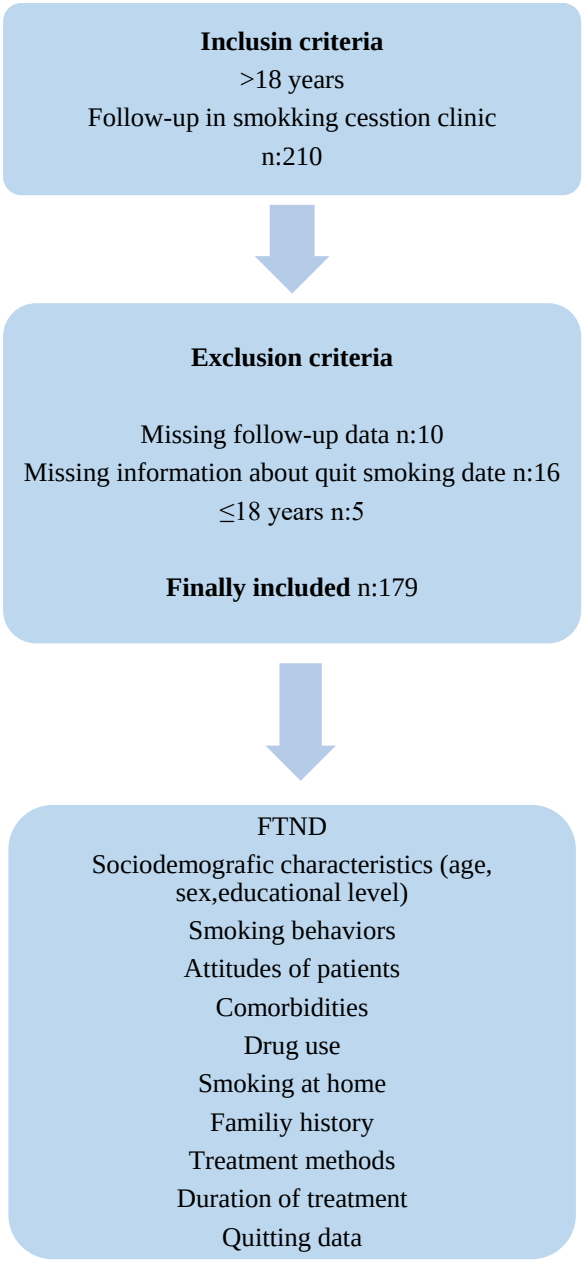

Figure 1. Design of the study

Statistical analysis: Data were analysed using Statistical Package for Social Sciences Version 21.0 (SPSS-21.0). In the evaluation of the data, the Kolmogorov-Smirnov test was used to compare whether the variables were normally distributed or not. The t test was used for normal distributions and Mann-Whitney U test was used for non-normally distributed variables. Chi-square analysis was used for categorical variables. Odds ratios were calculated by Cochran's and Mantel-Haenszel test. $p < 0.05$ was considered as statistically significant.

Results

Of the 210 individuals who applied to our outpatient clinic and completed the first year controls, 18-year-old patients (n = 5), who could not obtain information about the smoking cessation treatment response (n = 16) and who had incomplete information on their file (n = 10) were excluded from the study. As a result, 179 patients were included to the study. The mean age was 41.5 ± 13.7 years and 118 (65.6%) were male and 71 (39.7%) were married. 141 people (78.8%) were in high school and above. The highest rate of beginning to smoke was between the ages of 16 and 20 years (50.8%).

Behavioural treatment was applied to all individuals who applied to the outpatient clinic. If moderate and high levels of addiction were detected, pharmacological treatment was recommended.

At the end of twelve-month follow-up, 57 (31.8%) out of 179 people had quit smoking. The patients who quit smoking classified as Group 1 and those who did not as Group 2. There was no difference between Group 1 and Group 2, in terms of gender, age, working status and education level ($p = 0.356$, $p = 0.421$, $p = 0.603$, $p = 0.465$, respectively). Marital status was a protective factor for smoking cessation ($p = 0.049$ OR = 0.51 95% CI = 0.267-0.961) (Table 1).

Very low-moderate addicts (FTND score ≥ 5) and high-very high addicts (FTND score> 5) were compared in terms of smoking cessation status. There was no significant relationship between smoking cessation and FTND score ($p = 0.297$). (Table 2).

Table 1. The effect of socio-demografic characteristics on smoking cessation

		Group 1		Group 2		p
		N	%	N	%	
Gender	Male	36	63.2	82	67.2	0.356
	Female	21	36.8	40	32.8	
Age (year)	18-44	39	68.4	80	65.6	0.421
	≥45	18	31.7	42	34.4	
Working status	Employee	50	87.7	107	87	0.603
	Unemployeed	7	12.3	15	12.3	
Education status	Secondary scholl and under	11	19.3	27	22.1	0.465
	Hıgh school	12	21.1	34	27.9	
	University	34	59.6	61	50	
Marital status	Married	29	50.9	42	34.4	**0.049**
	Single	28	49.1	80	65.6	

Group 1: patients who quit smoking
Group 2: patients who do not quit smoking

Table 2. The relationship between FTND smoking cessation status

FTND	Group 1		Group 2		P
	N	%	N	%	
≤5	34	59.6	66	54.1	0.297
> 5	23	40.4	56	45.9	

FTND: Fagerström Test for Nicotine Dependence
Group 1: patients who quit smoking
Group 2: patients who do not quit smoking

In Group 1, 46 patients (80.7%) did not have comorbidity, while in Group 2, 46 patients (37.7%) had comorbidity. The presence of comorbidity in Group 1 was significantly lower than Group 2 ($p = 0.010$) (Table 3).

Smoking cessation rates were evaluated according to the treatment method used. In Group 1, the ratios of different treatment methods that were used were as follows; nicotine replacement therapy (NRT) and behavioural therapy 14% (n=8), varenicline and behavioural therapy 35.1% (n=20), bupropion and behavioural therapy 21.1% (n=12), combined treatment (varenicline, bupropion and behavioural therapy) 7% (n=4). When the distribution of treatment methods was examined, behavioural treatment with varenicline was the most common method in both groups. No significant difference was observed between the two groups in

terms of treatment methods used (p = 0.541) (Table 4).

Table 3. The presence of another smoker at home, the presence of comorbidity, previous attempts to quit smoking and the assessment of smoking cessation

		Group 1		Group 2		P
		N	%	N	%	
Another smoker in the home	Present	34	59,6	67	54,9	0.333
	Absent	23	40,4	55	45,1	
Comorbidity	Present	11	19,3	46	37,7	**0.010**
	Absent	46	80,7	76	62,3	
Attempt to quit	Yes	35	59,6	66	54,1	0.279
	No	23	40,4	56	45,9	

Group 1: patients who quit smoking
Group 2: patients who do not quit smoking

Table 4. Evaluation of methods used for cessation treatment

	Group 1		Group 2		P
	N	%	N	%	
NRT+ BT	8	14	23	18,9	0.541
Varenıclin + BT	20	35,1	44	36,1	
Bupropion + BT	12	21,1	31	25,4	
Behavioral therapy	13	22,8	16	13,1	
Combined treatment	4	7	8	6,6	

Group 1: patients who quit smoking
Group 2: patients who do not quit smoking
NRT: Nicotine replacement therapy
BT: Behavioral therapy

Discussion

The use of cigarettes and other tobacco products is an important public health problem and puts a huge burden on the national economy (WHO Report on the Global Tobacco Epidemic 2019). Today, smoking addiction, which is accepted as a disease, can be successfully treated with professional support (Karlıkaya et al., 2006). For this reason, WHO recommends that nicotine addicts should be encouraged to quit smoking and that the health system concentrates on smoking cessation methods and helps patients.

It has been reported that long-term success in self-quitters is less than 10% (Rigotti, 2012).

Studies have shown that outpatient clinics for smoking cessation increase the success rate (Carlson et all., 2002; Godfresten, 2008; Bize et al., 2010) One-year smoking cessation rate in our outpatient clinic was 31.8%. In similar studies conducted in our country, 1-year dropout rates were found to be 36.5% and 40.4% (Saglam, 2012; Argüder et al., 2013). In a Canadian study, the 1-year dropout rate was 43% (Carlson et al., 2002). The fact that the family of the individual was included in the social support program in this study, may be a factor that increases the success of quitting. In a Swiss study, the 1-year dropout rate was 27.1% (Bize et al., 2010).

In our study, the mean age at which the patients smoked for the first time was quite early (17.79 ± 4.52 years) and more than half of the patients (50.8%) had started smoking before the age of 20. Therefore an effective and correct approach towards smoking cessation in all age groups and especially in adolescents and early youth will be helpful to decrease the smoking rates and also quality of life and life expectancy could be increased. Beginning of smoking in adolescence period causes to become highly addictive and these individuals quit smoking more difficult. Tobacco Industry is therefore particularly target young people in their advertisements (Ozlu, 2002) Global Youth Tobacco Survey was assessed in 2017 at the age group of 13-15 years' students in a nationwide report on Turkey. 17.9% of students (23.2% of boys, 12.1% of girls) currently smoked a tobacco product, 7.7% (9.9% of boys and 5.3% of girls) were still smoking and 40.2% (46.8% of males and 33.1% of females) have tried a tobacco product at least once (Global Youth Tobacco Survey, Turkey, 2017).

In a study conducted by Şahbaz et al., it was shown that being married had a positive effect on smoking cessation success (Sahbaz et al., 2007). Similarly, in a study from England which had ten years' follow-up period, the rate of smoking cessation was significantly higher in the married individuals compared to those were unmarried (Chandola et al., 2004). In our study, in accordance with the literature, smoking cessation rate was higher in married patients. This may be due to the fact that married individuals are more determined to quit smoking because of their sense of responsibility and protection towards their families.

We found that absence of comorbidity had a positive effect on smoking cessation success. In the literature, there are studies reporting that patients with chronic disease quit smoking more difficult than those without. One study showed that patients with COPD quit smoking more difficult than healthy smokers (Solak et al., 2006; Onen et al., 2010). This made us think that patients with comorbidity may have been thought smoking cessation unnecessary because of their poor health. Contrary to these results, the presence of comorbidity has been

reported to have a positive effect on smoking cessation success in two studies (Can et al., 2004; Global Initiative for Chronic Obstructive Lung Disease (GOLD) Guidelines, 2011).

In a meta-analysis, it was reported that all forms nicotine replacement therapies increased smoking abstinence by 1.58 times (95% CI = 1.50-1.66) (Parsons et al., 2009). In another meta-analysis, the quit rate for six months was 22% in the nicotine patch group and 22% in the placebo group (Fiore et al., 1994). In our study, we did not only use NRT, but we found a one-year smoking cessation rate of 31.6% in individuals with NRT and behavioural therapy. The smoking cessation rate was 27% (Roddy, 2004) and 28% in two studies using bupropion treatment for six months (Hays et al., 2001). In a study comparing bupropion and nicotine patches, after 9 weeks of treatment, cessation rates were 32.5% for placebo, 41.4% for nicotine patches, 57.8% for bupropion, and 66.1% for bupropion and NRT combination. At the end of 1 year, cessation rates were 35.5% in the combination group, 30.3% in the bupropion group, 16.4% in the patch group, and 15.6% in the placebo group (Jorenby et al., 1999). In these studies, it was not indicated whether behavioural therapy was added or not to medical treatment (Jorenby et al., 1999; Hays et al., 2001; Roddy, 2004). In our study, quitting rates were 29.4% in the bupropion and behavioural treatment group, compared to quitting rate with bupropion in the literature and 31.6% in the NRT and behavioural treatment group. In a study comparing varenicline and NRT, one group received 1 mg varenicline twice a day and the other group received 21 mg transdermal patch. At the end of the twelve weeks of standard treatment regimen, quitting rates with varenicline were significantly higher than rates with standard transdermal nicotine treatment (56% and 43%, respectively) (Cahill et al., 2009). In our study, the difference between varenicline and NRT in terms of smoking cessation was not significant.

In a meta-analysis, it was shown that smoking cessation rate increased significantly for six months in parallel with the total minute duration in behaviour therapy contacts. The ratios of quitting were 14% in one to three minutes of counselling, 19% in 4-30 minutes of counselling and 27% in 31-90 minutes of counselling and 11% dropout rates was found among patients who did not take counselling therapy was given (Fiore, 2011). In our study, the rate of one-year smoking cessation was 31.7% in individuals who received only behavioural treatment.

In a placebo-controlled study which enrolled 51 patients the authors evaluated the use of combinations of bupropion SR and nicotine patch, and nicotine gum and behavioural therapy; they showed that addition of bupropion SR to the treatment regimen increased the reduction rate more than 50% (Evins et al., 2007). In our study, the rate of one-year smoking cessation was 41.7% in individuals using combined therapy. However, in our study, since the number of individuals who received combined treatment was very small, individuals were examined as one group heading, regardless of their drug differences.

Our study had some limitations. Retrospective method of the study may have been prevented the standardization of treatment. Because it is a single-center study, the patient population does not represent the whole population. In our study behavioural therapy applied to the patients was not uniform. So this could cause a confounding effect on the results. We had to exclude patients who had missing follow-up and quit smoking data. This limited the number of included patients.

As a result; Although there was no significant difference among the quitting methods used in our study, the quit rates (31.8%) were higher than the non-intervention quit rates reported in the literature. Patients with a high level of addiction benefit from smoking cessation methods. Being married in the smoking cessation process may positively affects the success of cessation. Since smoking is started mostly in adolescents and early youth, there is an urgent need for preventive measures to stop smoking and especially not to start.

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RESEARCH ARTICLE

Evaluation of Hepatitis B, Hepatitis C and HIV Seropositivity in Chronic Urticaria Patients

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Abstract

Objective: Many factors are responsible for the etiology of chronic urticaria. In this study, we aimed to determine the relationship between chronic urticaria and hepatitis B, hepatitis C and human immunodeficiency virus (HIV).

Methods: The study included patients with chronic urticaria who were admitted to our dermatology outpatient clinic between July 2018 and January 2019. Hepatitis B surface antibody (anti-HBs), hepatitis B surface antigen (HBsAg), hepatitis C antibody (anti-HCV) and human immunodeficiency virus antibody (anti-HIV) were recorded retrospectively.

Results: A total of 57 patients were included in the study. Of the 57 patients, 38 (66.7%) were female and 19 (33.3%) were male. The mean age of the patients was 41.2 ± 13.5 (minimum / maximum: 19-74). In thirty-two patients (56.1%), anti-HBs positivity was detected and in 25 (43.9%) negative. None of the patients had HBsAg positivity and HCV antibody positivity. Even though HIV antibody positivity was detected in one patient, the result of the validation test was reported as negative.

Conslusion: In our study, no relation was found between chronic urticaria and hepatitis B, hepatitis C and HIV. However, in patients with chronic urticaria, we think that there are more cases and multicenter studies to get an idea of whether screening for hepatitis and HIV should be done routinely.

Key words: Chronic urticaria, anti-HBs, anti-HCV, anti-HIV

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Introduction

Urticaria is defined as an itchy, erythematous and marked lesion that develops in the upper layers of the dermis due to vasodilatation and edema in the veins. According to the duration of lesions can be classified as acute and chronic. Chronic urticaria is defined as urticaria lesions lasting 6 weeks or longer. In patients with acute urticaria, it is easier to determine the causative agent and often food and drugs are responsible, but 40% of chronic urticaria cases cannot be detected. However, factors that play a role in the etiology of chronic urticaria include drugs, autoimmune diseases, infections, foods and psychogenic factors (Kaya and Akyol, 1999;

Ozdemir and Yazıcıoglu, 2012; Inci and Gunaydın, 2015; Sarac et al., 2018).

Chronic urticaria is seen in 0.5% of the population. The disease is seen in the adult age group, especially in middle-aged female patients. There is no clear data on the frequency of chronic urticaria in childhood (Ozdemir and Yazıcıoglu, 2012).

In the pathogenesis of urticaria, an increase in the regional vasodilatation and vascular permeability due to the effect of various vasoactive mediators, mainly histamine, plays an important role. The release of histamine and other vasoactive mediators is mediated by the effect of IgE antibody on the basophils or tissue mast cell surface with antigen, by the effect of C3a, C4a, C5a mediated by the complement system, or by histamine secreting agents released from mononuclear cells. In addition to histamine and other mediators, increased vascular permeability activates the plasma quinine system. Bradykinin as a result of activation of plasma kinin system also contributes to increased vasodilatation and permeability (Ozdemir and Yazıcıoglu, 2012). The hypothesis that chronic urticaria may be autoimmune for the first time has been proposed in 1960. In cases where the autologous serum skin test is positive, it is argued whether the autorespactivity is triggered by the autoantibodies or by the serum or plasma factor. The fact that basophils obtained from patients with chronic urticaria exhibit different responses in various tests compared to normal individuals suggested that a cellular defect in basophils may play a role in the pathogenesis. There is some information that chronic urticaria can be triggered by H. pylori, hepatitis and can be eliminated with the treatment of infectious agent. There are also case series associated with food and food preservatives. However, the evidence is not yet sufficient (Gungor and Akyol, 2014).

While many factors are considered responsible for the etiopathogenesis of chronic urticaria, recent studies have shown that infections have an important role in the etiology of urticaria. Upper respiratory tract infection, urinary tract infection, tooth infection, genital infection, chronic hepatitis B and C infections can be considered as a focus of infection (Kose et al., 2011).

Although the relationship between chronic urticaria and hepatitis has been known for a long time, the pathogenesis has not been fully understood. The accused mechanisms are the formation of degranulation of mast cells in the circulating antigens and complement activation via immune complexes (Inci and Gunaydın, 2015). Although there are cases in the literature where acute urticaria has been reported as the first sign of HIV infection, the frequency of chronic spontaneous urticaria in HIV-infected patients is not fully known (Iemoli et al., 2017).

In this study, we evaluated the results of anti-HBs, HBsAg, anti-HCV and anti-HIV in patients with chronic urticaria. It is aimed to compare with literature data.

Methods

The study included patients with chronic urticaria who were admitted to the dermatology clinic between July 2018 and January 2019. Ethics committee approval and patient approval could not be obtained because it is a retrospective study that should be performed in accordance with the principles of the Helsinki Declaration. Diagnosis of urticaria was made by clinical findings. Patients with erythematous, edematous papules and plaques on the trunk were diagnosed as urticaria. Patients with a duration of disease longer than 6 weeks were accepted as chronic urticaria. The patients were evaluated retrospectively and gender, age, HBsAg, anti-HBs, anti-HCV and anti-HIV results were recorded. Anti-HBs was considered positive for those above 10u/ML.

SPSS (Statistical Program for Social Sciences) 22 package program was used for statistical evaluation of the study. Normally distributed variables were shown as mean ± standard deviation.

Results

A total of 57 patients were included in the study. Of the 57 patients, 38 (66.7%) were female and 19 (33.3%) were male. The mean age of the patients was 41.2 ± 13.5 (minimum / maximum: 19-74). Thirty-two patients (56.1%) had anti-HBs positive and 25 (43.9%) patients were negative. In our patients, HBcAg (hepatitis core antigen) was not examined. Therefore, ın our study, it was not possible to differentiate patients with anti-HBs positivity from those with previous infection or vaccination-related positivity. Positivity for HBsAg, HCV antibody positive and none of the patients was not found. The results of the validation test were reported as negative even if HIV antibody positivity was detected in a patient (Table 1).

Table1. Anti-HBs, HBsAg, anti-HCV and anti-HIV results in patients with chronic urticaria

	Positive	Negative	Total
Anti-HBs			
Female	16	22	38
Male	9	10	19
HBsAg	-	57	57
Anti-HCV	-	57	57
Anti-HIV	1	56	57

Discussion

Urticaria is a disease characterized by erythematous, itchy papules and plaques in varying diameters. Chronic urticaria is defined if attacks last more than six weeks.

Urticaria is common in the community. 15-20% of people experience an attack of urticaria at least once in their lives. Acute urticaria is more common in children and young adults and chronic urticaria in middle-aged women. In our study, the majority of patients were female and middle-aged.

In chronic urticaria, many factors are responsible for release of certain mediators, especially histamine, from basophils and mast cells in immunological or non-immunological ways. But it is often difficult to determine the causative agent. Determining the etiology is important because it will affect the success of the treatment. Since all types of viral, bacterial, fungal and parasitic infections can cause urticaria, the focus of infection should be investigated in patients. Bacterial infections may be caused by respiratory tract, genitourinary system, dental, gastrointestinal tract. Hepatitis B and hepatitis C infections from viral infections are responsible for the etiology of chronic urticaria (Arican and Kutluk, 2005).

Viral hepatitis is a serious public health problem in our country and is frequently transmitted via blood. The incidence of hepatitis B virus in our country varies between 20.6% and 56.3%. The seroprevalence of the hepatitis C virus in our country is 0.3% - 1.8%. Hepatitis B and hepatitis C virus can cause cirrhosis, hepatocellular carcinoma and chronic hepatitis. In recent years, it has been suggested that viral hepatitis may be associated with many other autoimmune diseases such as mixed cryoglobulinemia, polyarteritis nodosa and Sjögren syndrome, other than liver findings. In addition, hepatitis C virus can be associated with many dermatological diseases such as pruritus, porphyria kutanea tarda, vasculitis, salivary gland lesions, lichen planus, necrolitic acral erythema (Balik, 1994a; Çakaloğlu, 1994b; Gül and Çevik, 1998; Bilen et al., 1999; Oztas et al., 2007; Inci and Gunaydın, 2015).

The relationship between chronic urticaria and viral hepatitis has been known for a long time. Some mechanisms such as insufficient clearance of immune complexes as a result of viral hepatitis-related macrophage function disorder, hepatitis virus stimulating the release of mediators from mast cells, immune complexes in circulation, release of antigens not related to HBV to circulating from infected hepatocytes are accused of etiopathogenesis. In addition, autoimmune mechanisms that play a role in the etiopathogenesis of chronic urticaria are thought to be triggered by agents with immunological effects such as HCV infection (Gul and Cevik, 1998; Dervis et al., 2001). In other studies, the pathophysiology of the disease is thought to be the formation of immune complexes in the blood and the accumulation of these immunocomplexes in the vessel wall. It is emphasized here that the process is associated with certain conditions in which antigen antibody complexes are well defined. Hepatitis B and C are the leading ones (Jones et al., 1983; Berg et al., 1988; Jerry et al., 1999). However, the opinions at the hypothesis level need to be supported.

Dermatological findings in the skin and mucous membranes of HIV-positive patients are important both in the diagnosis and progression of the disease. Recent studies have shown that herpes simplex virus infection, candidiasis, and seborrheic dermatitis are more common in HIV positive patients than in normal populations (Dilek and Saral, 2014). In addition, increased serum IgE levels can be detected in HIV infection. The increase in serum IgE levels is related to the imbalance between the T helper 1 and T helper 2 cytokine profile and the low peripheral TCD4 + T cell count caused by disease progression (Iemoli et al, 2017). Although there are many studies about the relationship between chronic urticaria and viral hepatitis in the literature, we did not find any comprehensive study examining the relationship between chronic urticaria and HIV positivity. In our study, a patient with chronic urticaria who was followed because of HIV positivity could not be detected. This situation may be related to the evaluation of patients with chronic urticaria during dermatology outpatient follow-up. Patients with HIV may also be evaluated in terms of chronic urticaria. Remarkably, chronic urticaria in the literature has been reported as the first sign of HIV infection (Friedman et al., 1995).

Iemoli et al, 56-year-old, HIV-positive, homosexual, men with a chronic urticaria treated with omalizumab reported (Iemoli et al, 2017).

In the study performed by İnci and Günaydın, 56 chronic urticaria cases were examined, 21 patients had anti-HBs positivity and 2 patients had HBsAg positivity. However, none of the patients had anti-HCV positivity (Inci and Gunaydın, 2015). Anti-HCV antibody positivity was found in 4 of 55 chronic urticaria patients in the study of Arinkal et al. (Arinkal et al., 2001). In another study, 36 patients with chronic urticaria were examined and none of them had anti-HCV antibody positivity. Therefore, the authors concluded that there is no need for anti-HCV investigation in patients with chronic urticaria who have no risk factor for HCV infection and normal liver function tests (Bilen et al., 1999). In a study by Erel et al., the seroprevalence of HBV and HCV in patients with chronic urticaria was similar to the normal population (Erel et al., 1999).

In some of the studies in the literature, patients with chronic urticaria reported higher rates of HCV antibody positivity than the normal population. Kazarava et al., 1996, in their study, 58 of 79 patients with chronic urticaria in 19 (24%) anti-HCV positivity has been found (Kanazawa et al., 1996).

In our study, none of the patients had HBsAg positivity and HCV antibody positivity. Even though HIV antibody positivity was detected in one patient, the result of the validation test was reported as negative. To determine the etiopathogenesis, whether HBsAg, anti-HCV and anti-HIV tests are to be performed in all patients should be evaluated in terms of cost effectiveness.

As a result, although studies in the literature that support the relationship between chronic urticaria and chronic hepatitis in our study none of the patients are hepatitis B, hepatitis C and HIV seropositivity could not be detected. However, in patients with chronic urticaria, we think that a wider range of cases and multi-center studies should be undertaken in order to have an idea of whether or not to have hepatitis and HIV screening routinely.

Ethics Committee Approval: Ethical committee approval was not obtained because the data were retrospectively reviewed in our study
Peer-review: Externally peer-reviewed.
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RESEARCH ARTICLE

Investigation of Hemogram, Endoscopy, Demographic Properties of Patients Applied by Fecal Occult Blood Test Screening

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Abstract

Objective: Colorectal cancer (CRC) is one of the most common cancers in the world. Fecal occult blood test (FOBT) is easy and commonly used test to its screening. The aim of this study was to investigate the demographic characteristics, hemogram results and endoscopy and outcome status of the patients who underwent FOBT on history and clinical suspicion and to contribute to the literature.

Methods: 400 patients who were admitted to the general surgery polyclinic between 01/12/2016 and 12/12/2017 in Ordu University Medical Faculty Training and Research Hospital were evaluated retrospectively. The data of 349 patients who underwent FOBT was reached. Age and sex, hemoglobin, hematocrit, ferritin levels, upper and lower gastrointestinal endoscopy results and pathology results of endoscopic biopsies were recorded.

Results: 38 (10.9%) with FOBT (+) and 311 (89.1%) with FOBT (-) of 349 patients were detected. There was no statistically significant difference between the two groups in terms of the average age (p = 0.27) and gender (p = 0.26). There was a significant difference between the two groups in terms of the rate of upper gastrointestinal endoscopy (p = 0.03). There was a significant difference between the two groups in terms of the rate of colonoscopy (p = 0.001). Malignancy was detected in 22,2% of FOBT (+) and in 1,63% of FOBT (-) group by colonoscopy. There was a statistically significant difference between the two groups in terms of disease diagnoses (p = 0.001). There was no statistically significant difference between the two groups in terms of *Helicobacter Pylori* (*H. pylori*) (p = 0.31) and intestinal metaplasia (p = 0.44). There was a statistically significant difference between the two groups in terms of Hb and Htc value (p= 0.05).

Conclusion: According to the results of FOBT, the application of endoscopy to the patients who are required, provide to detect the precancerous lesions as well as the malignancies of the upper and lower gastrointestinal tract. Good knowledge of the importance of this test will allow early detection of malignancy.

Keywords: Fecal occult blood test, Colorectal cancer, Endoscopy

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Introduction

Fecal occult blood test (FOBT) is a current screening method for the colorectal cancer (CRC). İt is based on the concept of detecting blood by the vascularized lesion (Carroll et al., 2014). Colorectal cancer is the third most common cancer in men and the second most common cancer in women worldwide (Torre et al., 2012). Therefore, screening and examination decision is important in early detection of suspected patients. FOBT is available, cheap and noninvasive test. On the other hand, anemia in peptic ulcer and gastric cancer may also be a sign of chronic bleeding, and in this respect, this test is a guide. *Helicobacter Pylori* (Hp) is the most common cause of many gastrointestinal diseases. More than half of the world's population is infected with Hp (Eusebi et al., 2014). İt is involved in the etiology of many gastrointestinal diseases as gastritis, peptic ulcer, functional dyspepsia, stubborn iron deficiency anemia, gastric mucosa-associated lymphoma (maltoma) and gastric adenocarcinoma (Lee et al.,2016; Bae et al., 2018; Adamu et al., 2010). In gastrointestinal system (GIS) diseases, simple complaints such as dyspepsia may be due to benign reasons such as *Helicobacter Pylori* and gastritis, yet dyspepsia, weight loss, secret or overt gastrointestinal bleeding may indicate a serious condition such as a gastric or colorectal malignancy (Ioannou et al., 2002). Symptom-based models have limitations, as they may present only at an advanced stage of the illness; furthermore, patients may ignore or not report symptoms. Therefore, only symptom-based examination and diagnosis are not sufficient. In this study, we aimed to investigate the demographic characteristics, hemoglobin, hematocrit, ferritin levels, *Helicobacter Pylori* existance, endoscopy and endoscopic pathology results in patients who underwent the FOBT on history and clinical suspicion.

Methods

A total of 400 patients who were admitted to the general surgery polyclinic between 01/12/2016 and 12/12/2017 in Ordu University Medical Faculty Training and Research Hospital were evaluated retrospectively. The data of 349 patients who underwent FOBT was reached. Age and sex, hemoglobin, hematocrit, ferritin levels, upper and lower GIS endoscopy results and pathology results of endoscopic biopsies were recorded. Detection of the fecal occult blood is made by the fecal immunohistochemical testing**.** Hemogram parameters were measured by Sysmex XN 1000 device. According to the World Health Organization Guidelines, the hemoglobin level below of 12 g/dl in women and below of 13 g/dl in men, the hematocrit level below of 36% in women and below of 39% in men, the serum ferritin level below of 50 mg/L and serum vitamin B12 level below of 200 pg/ml for both sexes were considered as low. Endoscopies of the patients were performed by the same specialist and biopsy specimens were evaluated by the same pathologist. Pathology specimens were stained with hematoxylin-eosin and modified giemsa method and examined by light microscopy.

The study was approved by Ordu University Ethics Committee.

Statistical Analysis:

İn our study, descriptive statistics were expressed as mean, standard deviation, minimum and maximum values for continuous variables and were expressed as numbers and percentages for categorical variables. Mann Whitney U test was performed for continuous variables. Chi-square test was used to determine the relationship between categorical variables. The statistical significance level was taken as 5% ($p = 0.05$) and the statistical package program (SPSS) was used for the calculations.

Results

The study included 349 patients which in 38 (10.9%) with FOBT (+) and 311 (89.1%) with FOBT (-). The mean age of the patients was 55.50 ± 14.74 (min 18-max 88). The mean age was 58.89 ± 12.89 (min 39-max 87) in FOBT (+) group and the mean age was 55.08 ± 14.99 (min 19-max 88) in FOBT (-) group. There was no statistically significant difference between the two groups in terms of the average age ($p = 0.27$).

Of the 349 patients, 239 (68.5%) were female and 110 (31.5%) were male. İn FOBT (+) group 23 (60.5%) were female and 15 (39.5%) were male. İn FOBT (-) group 216 (69.5%) were female and 95 (30.5%) were male. There was no significant difference between the two groups in terms of gender ($p = 0.26$).

The mean Hb value of the patients was 13.24 ± 1.78 (8.2-17.2) mg/dl. The mean Hb value was found to be 13.35 ± 1.84 (8.2-17.2) mg/dl in the FOBT (-) group and 12.35 ± 0.78 (11.1-13.2) mg/dl in FOBT (+) group. There was a statistically significant difference between the two groups in

terms of Hb value (p <0.05). The mean Htc value was %40,24 ± 4.72 (27.6-51.7). The mean Htc value was found to be % 40.18 ± 4.96 (27.76-51.7) in the FOBT (-) group and % 38.16 ± 2.74 (33.5-45.4) in FOBT (+) group. There was a statistically significant difference between the two groups in terms of Htc value (p <0.05).

When the patients were examined for anemia; it was detected in 49 (20.5%) female and 47 (42.7%) male patients. There was a statistically significant difference between two sexes in terms of anemia (p <0.001). Anemia was detected in 14 (36.8%) patients with (+) FOBT and in 82 (26.4%) patients with (-) FOBT. There was no statistically significant difference between the two groups in terms of anemia (p = 0.17). The mean ferritin value of the patients was 78.22 ± 74.85 (3.10-503) mg/L. There was no statistically significant difference between the groups in term of ferritin values (p> 0.05).

Helicobacter Pylori was detected in 3 patients of FOBT (+) in 15 patients of FOBT (-) group. There was no statistically significant difference between the two groups in terms of *H. pylori* presence (p = 0.31).

Intestinal metaplasia was not detected in any patients of FOBT (+) group but was detected in 7 patients of FOBT (-) group. There was no statistically significant difference between the two groups in terms of the presence of intestinal metaplasia (p = 0.44) (Table 1).

Table 1. Analysis of Age, Gender, Hemoglobin, Ferritin, *H. pylori*, Intestinal Metaplasia

Variables	FOBT (+) (n=38)	FOBT (-) (n=311)	P value
Age (mean ± sd)	58.89 ± 12.89	55.08 ± 14.99	0.27*
Gender			0.26*
Female	23	216	*
Male	15	95	
Hemoglobin (g/dl)	12.35 ± 0.78	13.35 ± 1.84	<0.05 *
Ferritin (mg/L)	59.54 ± 44.56	80.19 ± 77.18	0.40*
H. pylori	3	15	0.31*
Intestinal metaplasia	-	7	0.44*

*: Mann-Whitney U test was used. **: Chi-square test was used. P <0.05 was considered significant.

103 (29.5%) patients underwent upper GIS endoscopy and 246 (70.5%) did not. The rate of upper GIS endoscopy was 44.7% in patients with FOBT (+) (n = 17) and 27.6% in patients with FOBT (-) (n = 86). There was a significant difference between the two groups in terms of the rate of upper gastrointestinal endoscopy (p = 0.03).

Upper GIS endoscopy in FOBT (+) patients revealed antral gastritis in 7 patients, alkaline reflux gastritis in 1, pangastritis and bulbitis in 7, duodenal ulcers in 1 and normal in 1 patient. Upper GIS endoscopy in FOBT (-) patients revealed antral gastritis in 23 patients, alkaline reflux gastritis in 4, pangastritis in 5, esophagitis, pangastritis and bulbıtis in 18, pangastritis and bulbıtis in 21, alkaline reflux gastritis in 6, duodenal ulcer in 3, malignancy in 1 and normal in 1. There was no significant difference between endoscopic biopsy results between the two groups (p = 0.11) (Table 2).

79 (22.6%) of the patients underwent colonoscopy and 251 (77.7%) were not performed. The rate of colonoscopy was 47.4% in patients with FOBT (+) (n = 18) and 19.5% in patients with FOBT (-) (n = 61). There was a significant difference between the two groups in terms of the rate of colonoscopy (p <0.001).

18 of the patients (47.4%) with (+) FOBT underwent colonoscopy. Hemorrhoidal disease was detected in 12 patients, neoplastic adenoma in 2 patients and malignancy in 4 patients. 61 of the patients (%19,6) with (-) FOBT underwent colonoscopy. Hemorrhoidal disease was found in 23 patients, non-neoplastic adenoma in 4, neoplastic adenoma in 11, colitis in 8, malignancy in 1, diverticulosis in 1 and normal in 13 patients. There was a statistically significant difference between the two groups in terms of disease diagnoses (p <0.001) (Table 3).

Table 2. Comparative pathology results of upper gastrointestinal endoscopy

Pathological outcome	FOBT (+)	FOBT (-)
Antral gastritis	7	23
Alkaline reflux gastritis	1	10
Pangastritis and bulbitis	7	21
Duodenal ulcers	1	3
Normal	1	5
Pangastritis in 5	-	5
Esophagitis, pangastritis and bulbıtis	-	18
Malignancy	-	1
Total	n=17 44.7%	n= 86 27.6% (p = 0.11)

Table 3. Comparative pathology results of the colonoscopy

Pathological outcome	FOBT (+)	FOBT (-)
Hemorrhoidal disease	12	23
Neoplastic adenoma	2	11
Malignancy	4	1
Non-neoplastic adenoma	-	4
Colitis	-	8
Diverticulosis	-	1
Normal	-	13
	18 (47,4%)	61 (19,6%) (p <0.001)

Discussion

Colorectal cancer is the third most common cancer in men and the second most common cancer in women worldwide (Torre et al., 2012). Due to its slow progression from detectable precancerous lesions, initially symptoms may be nospecific and may be far from being diagnostic and early detection by screening test may be significantly life saver. Screening programs can reduce CRC mortality by 15–30% (Mandel et al., 2000; Von Karsa et al., 2013; Qaseem et al., 2012). FOBT is available, cheap and noninvasiv test for the CRC screening. The test sensitivity appears to be about 50 ([Lansdorp-Vogelaar et al.,2009). In our study, 22.6% of patients had colonoscopy. This may be due to the small number of patients with positive FOBT results. In addition, 47.4% of patients with FOBT positive and 19.6% of FOBT negative patients too underwent colonoscopy. It is expected to apply colonoscopy which is invasive but diagnostic, more specific and which allows for biopsy in FOBT positive patients. However, colonoscopy was decided even in up to 20% of patients with negative FOBT too. In this decision, other risk factors for colorectal cancer were taken into consideration by the clinician. To determine the CRC risk ratio, a number of CRC prediction models have been designed and validated in different settings (Williams et al.,2016; Rodriguez-Alonso et al., 2015). Other factors as age, sex and questionnaire-based information were used to make colonoscopy decision beside the FOBT (Joaquin et al., 2017). In our study, the mean age of the patients who underwent colonoscopy with negative FOBT was 54.95 ± and 37 (60.7%) were female and 24 (39.3%) were male of these patients. The mean age of the patients, with other risk factors, may have led to the decision of endoscopy.

In our study, 21 patients with positive FOBT refused to undergo upper gastrointestinal endoscopy. It is certain that this will cause a delay in the diagnosis of patients and a decrease in the percentage of possible malignancy diagnosis. Several complex factors play a role in affecting the patient's participation and sustained adherence. Screening rates have not increased as much as desired and remain at approximately 60% (Sabatino et al.,2015).

Similar and benign diagnoses were detected in upper GIS endoscopy of FOB positive and negative patients. In fact, one malignancy detection is in the FOB negative group. Already, it is known that clinical suspicion is more prominent than FOB in the suspicion of stomach and duodenal cancer. However, as known that atrophic gastritis and intestinal metaplasia are precancerous, early detection of these patients is important in terms of prognosis and mortality (Vannella et al., 2011).

In our study, *Helicobacter Pylori* (Hp) was detected in some of patients in FOBT (+) and both in FOBT (-). Hp is the causative agent of chronic gastric infections, and it has been estimated that at least half of the world's population is infected. A recent meta-analysis on the global prevalence of Hp infection has shown an overall prevalence of 44.3% (Zamani M et al., 2018). Socio-economic status, together with the level of urbanization and sanitation conditions, likely reflects the differences of Hp prevalence from country to country (Hooi et al., 2017). İt is involved in the etiology of many gastrointestinal diseases as gastritis, peptic ulcer, functional dyspepsia, stubborn iron deficiency anemia, gastric mucosa-associated lymphoma (maltoma) and gastric adenocarcinoma (Lee et al., 2016; Bae et al., 2018; Adamu et al., 2010). Hp as the main cause of gastritis confined to the antral mucosa and causes duodenal ulcer (Marshall. et al.,1984). Moreover, it has become clear that oxyntic atrophy is essential for gastric cancer (Testoni et al. 1987) including the carcinogenic effect of Hp infection (Uemura N et al., 2001).

Conclusion

Colorectal cancer is one of the most common cancers in the world. Therefore, importance should be given to its screening and detection. The FOBT is a useful and simple guide in colorectal cancer screening also in peptic ulcer or gastric cancer which can be followed by chronic bleeding. On the other hand, Hp should be detected and treated because it is accepted precancerous and it causes many benign and malignant diseases of the stomach. We observed that patients were not aware

of early cancer diagnosis by FOBT follow-up and endoscopy. Patients should be well informed about the FOBT importance about early detection of colorectal malignancy.

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RESEARCH ARTICLE

Antimicrobial Susceptibility Profiles and Prevalence of ESBLS among *Escherichia coli* Isolates Recovered from Clinical Specimens in Different Services

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Abstract

Objective: A retrospective analysis of the widely used antibiotics all susceptibility testing results from *Escherichia coli* cultured from clinical specimens private hospital from (January 2016 to November 2018) was performed.

Methods: The VITEK 2 Compact automated microbiology system is designed for automated rapid antimicrobial susceptibility testing and identification of clinically relevant bacteria. Minimum inhibitory concentration (MIC) results previously obtained in recent clinical isolates with well-defined in isolates with well-characterized resistance mechanisms with the microdilution method were re-interpreted for the susceptible, intermediate and resistant categories using the 2018 EUCAST breakpoints. Clinical samples are most commonly isolated from blood, sputum and urine samples.

Results: *Escherichia coli* isolates were highly resistant to ampicillin, cefuroxime and cefixime 79,16%, 60,41% and 58,33% respectively. Resistance rate of ceftriaxon was showed in 52,08%. When we compared to resistance of trimethoprim/sulfamethoxazole, *Escherichia coli* isolates showed 51,04% resistance rate. When it comes to the most sensitive antibiotics, sensitivity rate of fosfomycin, nitrofurantoin, ertapenem, imipenem, meropenem, gentamicin, and amikacin were 89,58%; 91,66%; 93,75%; 93,75%; 94,79%; 83,33%; 84,37% respectively.

Conclusion: Considering the antibiogram, fosfomycin, nitrofurantoin, ertapenem, imipenem, meropenem, gentamicin and amikacin should be preferred drugs for *E. coli* infection isolated from clinical samples.

Key words: *E. coli*, antibiotic sensitivity, clinical specimens

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Introduction

Escherichia coli (E. coli), a gram-negative bacteria normally residing in the intestinal tract, is among the most common pathogenic agents in humans and animals. It is classified into various pathotypes, causing in-testinal and extra-intestinal infections (Hammerum and Heuer, 2009). *E. coli* has shown an increasing in antimicrobial resistance to most antibiotics was isolated from human (Sherley et al., 2004). The β-lactam antibiotics including penicillins, cephalosporins, carbapenem

may act at several stages to prevent peptidoglycan synthesis (Jones et al., 2000). The incidence of infections caused by *Enterobacteriaceae* producing extended-spectrum beta lactamase (ESBL) has increased rapidly in the last 5 to10 years, mainly attributed to the successful distribution of CTX-M enzymes among *E. coli* causing urinary tract and bacteremic infections (Kang et al., 2012).

The aim of this study was to determine the characteristics and patterns of antibiotic resistance among isolates of *E. coli* recovered from clinical specimens in Giresun province.

Methods

Bacterial isolates

The ninety-six *E. coli* were isolated from clinical specimens from different services in private hospital. Bacterial isolates were identified and then commonly used for AST (Antimicrobial Susceptibility Testing) in clinical laboratories will therefore have to incorporate these criteria in their instruments to meet the needs of European microbiology laboratories according to standard methods described by (Cappuccino and Sherman, 2004). All isolates were obtained from patients at intensive care units. In total, ninety-six *E. coli* were isolated from various clinical samples and detected by the VITEK 2 (bioMérieux) at the microbiology laboratory of our hospital between from January in 2016 to December in 2018 (Ling et al., 2001). The data obtained were evaluated as numbers and percentages. Comments were made accordingly. It has taken the necessary permission from the outhorities.

Antibiogram profile of E. coli

Minimum Inhibitory Concentration (MIC) results previously obtained in recent clinical isolates with well-defined in isolates with well-characterized resistance mechanisms with microdilution method were re-interpreted using the 2018 EUCAST breakpoints. Fifteen different antibiotics were used. Antibiotics tested in AST-N327 (bioMérieux) card included ampicillin (AM), tazobactam/piperacillin (TPZ), cefuroxime (CXM), cefixime (CFM), ceftazidime (CAZ), ceftriaxone (CRO), ertapenem (ETP), imipenem (IMI), meropenem (MEM), amikacin (AK), gentamicin (GN), ciprofloxacin (CIP), fosfomycin (FOS), nitrofurantoin (F), trimethoprim-sulfamethoxazole (SXT).

Detection of ESBL

VITEK 2 system with the antimicrobial susceptibility extend card AST-N327 (bioMérieux) card was designed to perform both screening and confirmatory tests for phenotypic detection of ESBL on the same plate. The use of several antimicrobial agents increases the sensitivity of ESBL detection (Sorlózano et. al., 2005).

Multiple Antibiotic Resistance (MAR) index

MAR index values were tested for according to (Matyar et al., 2008).

Results

The results of resistance pattern of *E. coli* isolates in our locality to antimicrobial agents showed that the ninety-six *E. coli* strains tested against fifteen antimicrobial agents in Table 1. *E. coli* isolates were highly resistant to AM, CXM and CFM 79,16%, 60,41% and 58,33% respectively. Resistance rate of CRO was showed in 52,08%. When we compared to resistance of SXT, *E. coli* isolates showed 48,95% resistance rate. When it comes to the most sensitive antibiotics, sensitivity rate of FOS, F, ETP, IMI, MEM, GN and AK were 89,58%; 91,66%; 93,75%; 93,75%; 94,79%; 83,33%; 84,37% respectively as is illustrated in Table 1. Table 2 shows the antimicrobial susceptibility of all *E. coli* isolated from urine, blood, and sputum. Of the total *E. coli* isolates, 50 (%52) isolates were ESBL producers and 46 (48%) isolates were non-ESBL producers in Table 2.

Discussion

In our study, when we compared to resistance of AM, *E. coli* isolates showed high antibiotic resistance with 79,16% AM. Some researchers have reported resistance rate AM from 88,4% to 16,5 % to *E. coli* in clinical samples (Tadesse et al., 2012; Niranjan and Malini., 2014). Our results were similar to Weissman et al. (2015) who also reported that AM resistance rate of *E. coli* 78%.

The result of resistance CXM rate, *E. coli* isolates showed high antibiotic resistance with 60,41% CXM. Previous researchers have reported resistance rate CXM from 100% to 60% to *E. coli* in clinical samples (Ugwu et al., 2017; Cheema et al., 2018).

In this study, resistance rate of CFM, *E. coli* isolates showed high antibiotic resistance with 58,33% CFM. Some researchers have reported resistance rate CFM from 94% to 40% to *E. coli* in clinical samples (Sah et al., 2016; Tasleem et al., 2018). Our results were like Cheema et al.,2018

who also reported that CFM resistance rate of *E. coli* 61%.

The resistance rate of CRO showed 52,08%. Many researchers were reported that resistance rate

Table 1. Antibiotic suspectibility pattern of ninety-six *E. coli* isolated from urine, sputum and blood samples

Antibiotics	Resistance	Intermediate	Sensitive
AM	76 (79,16%)	-	20(20,83%)
CXM	58(60,41%)	-	38(39,58%)
CFM	56(58,33%)	-	40(41,66%)
CRO	50(52,08%)	3(3,12%)	43(44,79%)
SXT	47(48,95%)	-	49(%51,04)
CAZ	43(44,79%)	8(8,33%)	45(46,87%)
CIP	40(41,66%)	2(2,08%)	54(56,25%)
TPZ	23(23,95%)	22(22,91%)	51(53,12%)
GN	14(14,58%)	2(2,08%)	80(83,33%)
FOS	10(10,41%)	-	86(89,58%)
F	8(8,33%)	-	88(91,66%)
ETP	6(6,25%)	-	90(93,75%)
IMI	2(2,08%)	4(4,16%)	90(93,75%)
MEM	2(2,08%)	3(3,12%)	91(94,79%)
AK	2(2,08%)	14(14,58%)	80(83,33%)

Abrevations; AM, Ampicillin, CXM, Cefuroxime; CFM, Cefixime, CRO, Ceftriaxon SXT; Trimethoprim sulfamethoxazole, CAZ; Ceftazidim, CIP; Ciprofloxacin, TPZ; Piperacilin-Tazobactam, GN; Gentamicin, FOS, Fosfomycin, F; Nitrofurantoin, ETP, Ertapenem; IMI; Imipenem; MEM; Meropenem, AK; Amikacin

Table 2: Distribution of ninety-six *E. coli* clinical samples, sexuality, source, MAR Index and ESBL Producers

Name of Clinic	Number of samples	Source of isolates	Sexuality F/M	ESBL Producers	MAR İndex
Infectious Diseases	49	Urine	35F/14M	30P/19N	0(7isl);0,07(4isl);0,13(2isl);0.2(3isl);0,27(5isl);0,53(3isl);0,33(4isl);0,6(3isl);0,8;0,47(8isl); 0,4(6isl); 0,67(3isl);0.87
Internal medicine	11	Urine	7F/4M	4P/7N	0; 0,2; 0,07(2isl); 0,13(2 isl); 0,47(3isl); 0,53(2isl);
Child Health and Diseases	12	Urine	4F/8M	3P/9N	0(2isl); 0,07(2isl);0,13(2isl); 0,2(2isl); 0,4(2isl); 0,53; 0,47
Urology	6	Urine	2F/4M	4P/2N	0,07(2isl);0,53(2isl); 0,47; 0,4
Chest Diseases	7	5 Sputum 1Urine 1Blood	3F/4M	6P/1N	0,47, 0,53(3isl); 0,33(2isl); 0,27
Anesthesia and Reanimation	2	Urine	2M	1P/1N	0,07;0,047
Physical Medicine and Rehabilitation	1	Urine	1F	1N	0,13
Gynocology	1	Urine	1F	1N	0
Cardiology	3	1Blood 2Urine	2F/1M	2N	0, 0,33;0,93
Norologia	1	Urine	1M	1N	0
Pediatric Surgery	1	Urine	1F	1N	0,13
Total	96	89 Urine, 5 Sputum, 2 Blood		50P (%52) 46N(%48)	63 (66%) ≥ 0.2 33 (34%) <0.2

MAR, Multiple Antibiotic Resistance Index, isl; ısolates, +; ESBL Producing-; Non-ESBL Producing F; Female, M; Male

of CRO varied from 41.6% to 91.8% (Park et al.,2012; Ayatollahi et al.,2013). Our results were higher than Ayatollahi et al. (2013) who also reported that CRO resistance rate of *E. coli* 41,6%.

In our study, when we compared to resistance of SXT, *E. coli* isolates showed high antibiotic resistance with 48,95% SXT. In the previous researchers have reported resistance rate SXT from 34% to 16,1% to *E. coli* in clinical samples (Karlowsky et al., 2002; Guneysel et al., 2009).

When it comes to the most sensitive antibiotics, sensitivity rate of FOS, F, ETP, IMI, MEM, GN and AK were 89,58%; 91,66%; 93,75%; 93,75%; 94,79%; 83,33%; 84,37% respectively. Some researchers have reported sensitive rate FOS from 100% to 86% to *E. coli* in clinical samples (Ayub et al., 2016; Lawhale and Naikwade., 2017; Wagle et al., 2018).

Our results were similar to Ouizdi et al. (2018) who also reported that FOS sensitivity rate of *E. coli* 92%. Some researchers have reported sensitive rate F from 100% to 86% to *E. coli* in clinical samples (Lawhale and Naikwade, 2017; Ouizdi et al., 2018). Our results were similar to Ouizdi et al. (2018) who also reported that F sensitivity rate of *E. coli* 92%. Naber et al., (2010) repoprted that a positive urine culture was found in 74.6%, and *E. coli* was most frequent (76.7%) with the highest rate of susceptibility to fosfomycin (98.1%). Similar findings of high susceptibility to nitrofurantoin and fosfomycin shown by Gupta et al. (2013), Fajfr et al. (2017) which is supportive to this study (Gupta et al., 2013; Dash et al., 2013; Fajfr et al., 2017).

The sensitivity rate of TPZ was 53,12%. In previous researchers have reported sensitive rate TPZ from 57.9 % to 9.2% to *E. coli* in clinical samples (Ghafur et al.,2012; Rugini et al.,2015). Our results were similar to Ghafur et al.,2012 who also reported that TPZ resistance rate of *E. coli* 57.9%.

The susceptibility rate of ertapenem from 97.6% to 100% in different countries like Turkey, Estonia, Latvia, Lithuania, Portugal, Romania, Switzerland. and UK (Hawser et al., 2012). Many researchers have reported sensitivity rate ETP from 100% to 14,8% to *E. coli* in clinical samples (Malhotra et al., 2016; Devrim et al., 2018).

Unlike our study, no resistance to IMI was observed by Al-salamy (2012), Malhotra et al. (2016). Some researchers have reported that MEM sensitivity rate to *E. coli* in clinical samples (Mulla et al.,2011; Fernando et al.,2017).

Many researchers reported ESBL activity in *E. coli* strains, Albayrak and Kaya (2009), Eryilmaz et al. (2010) and Mumcuoglu et al. (2004) 19%, 20% and 6% respectively. Guducuoglu et al. (2007) reported the samples according to the origin as policlinics or clinics and showed the ESBL activity as 18% and 47% respectively in *E. coli* strains.

In the present study, 63 (64%) of the isolates showed Multiple Antibiotic Resistance three to fourteen antibiotics. All of the isolates showed resistance to at least one antibiotic. Malhotra et al. (2016) reported that out of 41 MDR isolates, maximum numbers of MDR strains were from urine (43.9%) followed by pus (41.5%), blood (8.3%), fluid (5.6%) and sputum (2.8%) samples (Malhotra et al., 2016). Niranjan & Malini also observed that majority of *E. coli* isolates (76.5%) from urine samples were multidrug resistant like ours.

Conclusion

In this study, it has been shown that *E. coli* in different clinical specimens have experienced high resistance to AM, CXM, CFM and CRO. Some antibiotics show low resistance such as IMI, MEM and AK. An effective national and state level antibiotic policy should be framed for preserving the effectiveness of antibiotics and prevent the emergence of resistance.

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RESEARCH ARTICLE

Method Verification of Inhouse Real-time Polymerase Chain Reaction for Detection of Leishmania Species

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Abstract

Objective: Leishmaniasis is a vector-borne disease caused by many *Leishmania* species which can infect both humans and other mammals. Turkey has special epidemiologic importance in terms of this disease due to being located at the junction of Asia and Europe and containing seven geographical regions with environmental and ecologic differences. Microscopic evaluation may miss diagnosis in cases with low levels of parasitemia. The culture method is not chosen by many laboratories due to being laborious preparation of media and results taking a long time. Molecular methods may assist in diagnosis especially with low parasitemia levels and provide results in a short time, so they have begun to be chosen more often currently. This study aimed to verify the method for inhouse real-time PCR using primers for ITS-1 gene region for routine molecular diagnosis of leishmaniasis.

Methods: After parasite counting from *Leishmania infantum* strain, which was carried out on Novy-MacNeal-Nicolle (NNN) medium in our laboratory, DNA extraction was performed with a commercial kit (QIAamp DNA Blood Mini kit, Qiagen, Germany) according to the manufacturer's recommendations. The number of copies in the reaction was determined from DNA sample and serial dilutions of 1/10 were prepared. Then, inhouse real-time PCR method was applied using primers targeting the ITS-1 gene region to determine limit of detection and high and low positivity rates. Then accuracy and precision studies were performed with these samples for method verification. Amplification was performed with a Light Cycler 96 (Roche, France) device. Results were evaluated with amplification curve analysis. The variation coefficients were calculated via accuracy and precision studies from the obtained results.

Results: The parasite counts of *Leishmania infantum* strain were determined as 17,000 promastigote/ml. With using 400 µl of this sample (6,800 copies), DNA obtained in 100 µl elution buffer. Using inhouse real-time PCR amplification, the detection limit for 2 µl DNA (136 copies) was determined as 10^{-3} dilution (0.136 copies/reaction). The high positivity rate was determined as dilution above 2 log10 of limit of detection's and the low positivity rate was determined as dilution above 1 log10 of limit of detections. Efficiency of the method was measured with a regression curve for CT values. The standard curve obtained according to CT values and equivalent promastigote counts was linear (slope: -4.097). There was a significant correlation coefficient found between mean CT values and *Leishmania infantum* DNA concentrations (R=0.99).

Conclusion: Our study determined the variation coefficient for the inhouse real-time PCR method was below 15%, confirming it is appropriate for use in our laboratory for routine molecular diagnosis of *Leishmania* spp.

Key words: *Inhouse realtime PCR, method verification, Leishmania spp.*

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Introduction

Leishmaniasis is a vector-borne disease caused by many *Leishmania* species which can infect both humans and other mammals (Galluzzi L, et al., 2018). It is accepted as one of the most common infectious diseases following HIV/AIDS, tuberculosis and malaria (Rezvan H, et al., 2017). According to the World Health Organization, more than 350 million people in 89 countries are at risk in terms of this disease (Rezvan, et al., 2017; Moreira, et al., 2018; Akhlagh, et al., 2019). Annually there are nearly 0.2 to 0.4 million visceral leishmaniasis cases and 0.7 to 1.2 million cutaneous leishmaniasis cases reported (Rezvan, et al., 2017; León, et al., 2017; Galluzzi, et al., 2018). Turkey has special epidemiologic importance in terms of this disease due to being located at the junction of Asia and Europe and containing seven geographical regions with environmental and ecologic differences (Ozensoy Toz, et al., 2013). Linked to *Leishmania* species and host features, the disease may occur with a variety of clinical appearances from asymptomatic infection, cutaneous leishmaniasis progressing with skin involvement, visceral leishmaniasis progressing with internal organ involvement, and mucocutaneous leishmaniasis progressing with mucosa and skin involvement (León, et al., 2017; Galluzzi, et al., 2018; Moreira, et al., 2018; Akhlagh, et al., 2019). Cutaneous leishmaniasis is the most commonly observed clinical form (Moreira, et al., 2018). There are two forms of cutaneous leishmaniasis: Anthroponotic cutaneous leishmaniasis caused by *Leishmania tropica* (*L. tropica*) as agent and zoonotic cutaneous leishmaniasis caused by *Leishmania major* (L. major) as agent (Rezvan, et al., 2017; Akhlagh, et al., 2019). In Turkey, anthroponotic cutaneous leishmaniasis caused by *L. tropica* is endemic in the southeast Anatolia, eastern Mediterranean and Aegean regions. In addition to *L. tropica*, in southeast Anatolia there are human cutaneous leishmaniasis cases reported caused by *Leishmania infantum* (*L. infantum*) as agent. The disease is endemic in countries along Turkey's southern border (Syria, Iraq, and Iran) (Ozensoy Toz, et al., 2013). The most serious form of the disease is visceral leishmaniasis, also known as kala-azar. The etiologic agent is mostly *L. infantum*, and a *Leishmania donovani* (*L. donovani*) complex comprising *L. donovani* and *Leishmania chagasi (L. chagasi)* (Rezvan, et al., 2017). In recent times, in addition to visceral leishmaniasis cases caused by *L. tropica*, cutaneous leishmaniasis cases caused by *L. donovani* and *L. infantum* are reported. Due to increased travel possibilities, climate changes, and changes in socioeconomic and sociopolitical conditions, new species may be observed in our country (Sirekbasan S, et al., 2109). Visceral leishmaniasis is endemic to the Mediterranean, Aegean, Marmara and Black Sea regions, while sporadic cases are observed in other regions (Ozensoy Toz S, et al., 2013). The mucocutaneous form (espundia) occurs due to *Leishmania brazilensis (L. brazilensis*), *Leishmania panamensis (L. panamensis)*, and *Leishmania guyanensis (L. guyanensis)* species in the New World, while frequently *L. infantum* and *L. donovani* species are agents in other regions. The most significant symptoms of cutaneous leishmaniasis is ulcerative skin lesions in bite region and multiple nonulcerative nodules. The symptoms of visceral leishmaniasis include fever, skin pigmentation, loss of appetite, fatigue, hepatosplenomegaly, lymphadenopathy, pancytopenia, weight loss and death. Due to showing similarities to many diseases, differential diagnosis is very important. While cutaneous leishmaniasis may be confused with leprosy, skin tuberculosis and malignancies, visceral leishmaniasis may be confused with malaria and schistosomiasis (Rezvan, et al., 2017).

Methods

After parasite counting from *L. infantum* strain, which was carried out on Novy-MacNeal-Nicolle (NNN) medium in our laboratory, DNA extraction was performed with a commercial kit (QIAamp DNA Blood Mini kit, Qiagen, Germany) according to the manufacturer's recommendations. The number of copies in the reaction was determined from DNA sample and serial dilutions of 1/10 were prepared. Then, inhouse real-time PCR method was applied using primers targeting the ITS-1 gene region (Table 1) to determine limit of detection and high and low positivity rates. The PCR mix rates for inhouse real-time PCR method are given in Table 2 and amplification conditions are given in Table 3. Then accuracy and precision studies were performed on these samples for method verification. As negative control, RNAase/DNAse free water was used. For qualitative tests, the accuracy studies was performed with three high and low positive and negative samples. Precision studies were applied in two ways, inter assay and intra assay study. While in intra assay precision studies, three high and three low positive samples were studied at one time, in inter assay precision studies one high and one low positive samples were studied at three different days. Amplification was performed with Light

Cycler 96 (Roche, France) device. The results were evaluated with amplification curve analysis. The variation coefficients (CV%) were calculated in accuracy and precision studies from the obtained results. For evaluation of method verification results, Cumitech 31A 2009 was taken as reference (Clark, et al., 2009).

Table 1. Inhouse real-time PCR primers

Forward primer LSGITS1-F1	5'-CATTTTCCGATGATTACAC-3'
Reverse primer LSGITS1-R1	5'-CGTTATGTGAGCCGTTATC-3'

Table 2. Inhouse real-time PCR mix

H_20	6 µl
Syber Green Master mix	10 µl
Primer F (LSGITS1-F1)	1 µl
Primer R (LSGITS1-R1)	1 µl
DNA	2 µl
Total volume	20 µl

Table 3. Amplification conditions of inhouse real-time PCR

95 ^{0}C	5 min	Initial denaturation
95 ^{0}C - 15 sec	45 cycles	
55 ^{0}C - 40 sec		
72 ^{0}C - 60 sec		
72 ^{0}C	2 min	Final extention

Results

The parasite counts of *L. infantum* strain were determined as 17,000 promastigote/ml. With using 400 µl of this sample (6,800 copies), DNA obtained in 100 µl elution buffer. The study results for inhouse real-time PCR serial dilutions are given in Table 4. Using inhouse real-time PCR amplification, the limit of detection for 2 µl DNA (136 copies) was determined as 10^{-3} dilution (0.136 copies/reaction). The high positivity rate was determined as dilution above 2 log10 of limit of detection's and the low positivity rate was determined as dilution above 1 log10 of limit of detection's. The verification study results for inhouse real-time PCR method are presented in Table 5. To calculate variation coefficient, firstly standard deviation and mean were calculated, then coefficient of variation (CV%) was obtained by dividing standard deviation to mean. Accordingly, when variation coefficients are calculated for both accuracy and precision studies, it was determined to be below 15%. The amplification curves for limit of detection study of inhouse real-time PCR method are given in Figure 1. Efficiency of the method was measured with a regression curve for CT values. The standard curve obtained according to CT values and equivalent promastigote counts was linear (slope: -4.097) (Figure 2). There was a significant correlation coefficient between mean CT values and *L. infantum* DNA concentrations (R=0.99).

Table 4. Serial dilution results of inhouse real-time PCR

Dilution Rate	**Copy number**
10 ^{0} dilution	136 copy
10 $^{-1}$ dilution (high positive)	13,6 copy
10 $^{-2}$ dilution (low positive)	1,36 copy
10 $^{-3}$ dilution (limit of detection)	**0,136 copy**

Table 5. The results of inhouse real-time PCR method verification study

	Accuracy Study	**Precision Study (intra assay)**	**Precision Study (inter assay)**
High positive (%CV)	0	0,0088	0,0230
Low positive (%CV)	0	0,0136	0,0136

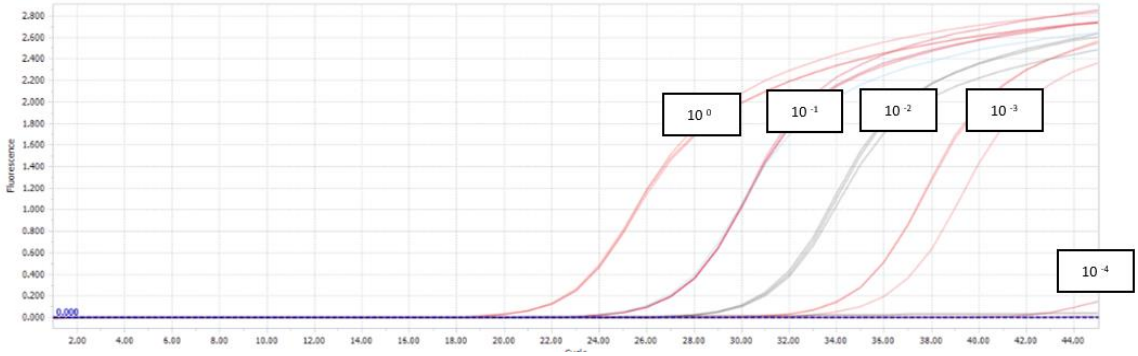

Figure 1. Amplification curves of limit of detection study of the inhouse real-time PCR method

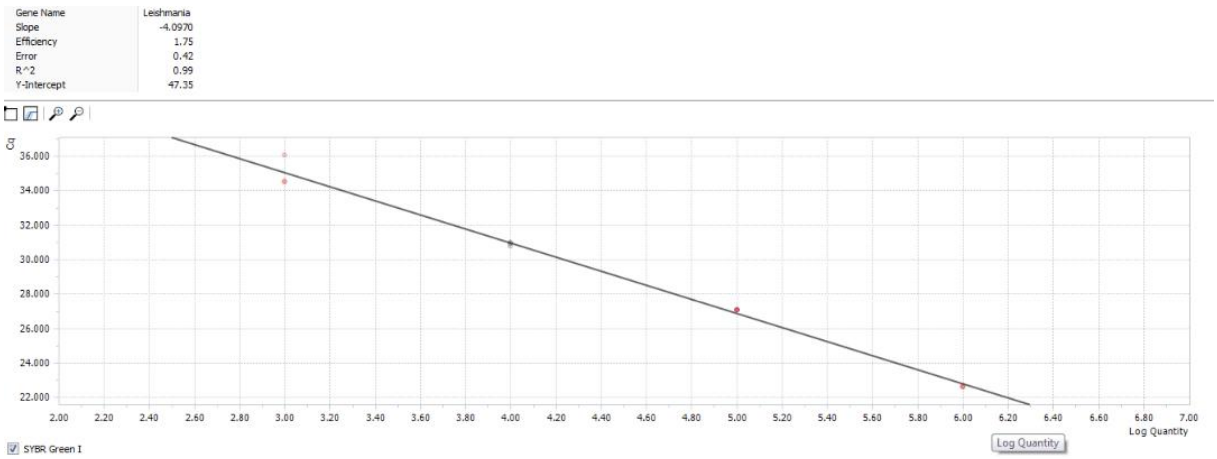

Figure 2. Plot of threshold cycle (CT) versus log promastigote number obtained from inhouse real-time PCR

Discussion

For diagnosis of the disease microscopy, culture, serologic and molecular methods are used (Mary, et al., 2004; Rezvan, et al., 2017; Moreira, et al., 2018; Soofi Abadi, et al., 2019; Deepachandi, et al., 2019). Microscopy is still accepted as the gold standard method. Parasite culture with NNN media may be used for supporting diagnosis. However, preparation of media is laborious, requires technical expertise, tends toward contamination, and it takes a long time (de Vries, et al., 2015; Deepachandi, et al., 2019). The sensitivity of parasite culture and accompanying microscopic investigation is reported to increase to up to 85% (Rezvan, et al., 2017). With the light microscopy, *Leishmania* species cannot be distinguished morphologically from each other; however, species differentiation is very important for patient management (de Vries, et al., 2015; Rezvan, et al., 2017). Additionally, the use of light microscopy with in vitro culture methods is limited for chronic, atypical or treated lesions (Deepachandi, et al., 2019). Though the specificity for microscopic investigation in routine diagnosis of cutaneous leishmaniasis especially is high (100%), but sensitivity is low. The sensitivity of the method varies from 40-74.4%, depending on condition of skin lesion from which sample is taken, localization from which sample is taken and expertise of personnel performing microscopic examination. As a result, more sensitive methods such as PCR were developed as alternatives for diagnosis of this disease (Rezvan, et al., 2017; León, et al., 2017). The serologic diagnosis of visceral leishmaniasis is based on methods such as indirect fluorescent antibodies, ELISA, western blot, lateral flow and direct agglutination test. However, these methods are not widely used for cutaneous leishmaniasis diagnosis due to weak humoral response caused by infection and low sensitivity (de Vries, et al., 2015). Molecular techniques such as PCR and real-time PCR can distinguish parasite species (Soofi Abadi MF, et al., 2019). Definition of parasite species is very important to understand the disease epidemiology (Ozensoy Toz, et al., 2013). Previously, isoenzyme analysis was used for species differentiation. This method is based on differences in electrophoretic mobilities of enzymes isolated from *Leishmania* species. However, due to high cost, time required and need for large amounts of cultured promastigote, it is only applied in a few reference laboratories. As a result, alternative typing methods have been developed especially based on the genetic features of the parasite (de Vries, et al., 2015; Rezvan, et al., 2017). Molecular methods are reported to have better sensitivity and specificity that traditional diagnostic methods (de Vries, et al., 2015). Methods such as conventional single-stage PCR, nested PCR, PCR-RFLP and quantitative real-time PCR (qPCR) are commonly used. Though nested PCR is one of the most sensitive PCR techniques, an important limitation is false positivity due to possible cross contamination (Hernández, et al., 2014; Deepachandi, et al., 2019). QPCR is a method that is more rapid compared method to traditional PCR, repeatable, has high sensitivity and allows quantitative testing (Paiva-Cavalcanti, et al., 2010; Hernández, 2014; Galluzzi, et al., 2018; Moreira, et al., 2018; Antonia, et al., 2018). As reaction tubes do not need to be opened after amplification, cross contamination is largely reduced (Galluzzi, et al., 2018). Real-time PCR is based on use of stains or fluorescent probes allowing observation of the amplified product (Paiva-Cavalcanti, et al., 2010; Galluzzi, et al., 2018). With this aim, SYBR Green is a commonly used stain and binds nonspecifically to DNA double

strand produced during amplification (Paiva-Cavalcanti, et al., 2010). SYBR Green based analyses and HRM analyses allow possibility of differentiating *Leishmania* subgenus or species according to melting curves obtained for each sample (Moreira, et al., 2018). Another way to produce fluorescence is to use a probe targeting the internal sequence region required for amplification (e.g., TaqMan probe). During amplification TaqMan degrades and leaves a light-emitting reporter. Light emission analysis is made by a light signal detector forming a graph with absorbance obtained from each PCR cycle. The produced signal reflects the amount of product produced (Paiva-Cavalcanti, et al., 2010). In terms of cost, Taqman analysis has higher cost. Additionally, due to allowing the possibility of identifying multiplex reactions, the test costs may be accepted as relatively lower (Moreira, et al., 2018). The point when the fluorescence emission of sample exceeds a threshold value is called cycle threshold (CT) and is related to amount of amplified DNA. Along the amplification curve, the CT of each analyzed sample can be checked and decisions made about whether it is positive or not. Values above 35 CT may cause false positive results (Paiva-Cavalcanti, et al., 2010). The reasons for this situation are considered to be samples containing an inhibitor or low parasitemia levels. After treatment, a similar situation may be encountered with samples. As a result, evaluations should be made carefully. For example, the inhibitor content problem may be solved by studying diluted DNA, while the increase in DNA amounts in samples with low parasitemia rates may reduce CT values. The evaluation results in our study accepted samples with CT value above 38 as being negative. The sensitivity of molecular methods varies linked to the selection of target gene region (Mary, et al., 2004; Galluzzi, et al., 2018). With this aim, many target genes have been defined. The most commonly used multiple copy genes such as kDNA, ITS-1 and HSP70 genes have advantages such as low detection limits and potential to differentiate species (Hernández, et al., 2014; de Vries, et al., 2015; Rezvan, et al., 2017; Moreira, et al., 2018; Antonia, et al., 2018). KDNA can be determined up to 10,000 copies, 18S rRNA has 166 copies, ITS-1 has 40 to 200 copies and HSP70 has 5 to 7 copies. When evaluated in terms of detection limits, multiple copy genes may reveal significant degrees of variability between parasite species, strains, life cycle stages and clinical isolates. Single copy genes tend to be found in more stable regions of genome; however, detection limits are generally higher compared to multicopy genes. Single copy genes are glucose phosphate isomerase (GPI), glucose 6 phosphate dehydrogenase (G6PD), superoxide dismutase 1 (SODB1), arginine permease (AAP3) and DNA polymerase (Antonia, et al., 2018). Though there are many studies published about qPCR, each laboratory applies their own method's as there are no determined general protocols. There are very few studies about comparisons between laboratories, especially. There is requirement for studies on this topic (de Vries, et al., 2015). Additionally, evaluation of analytic performance to confirm a diagnostic method requires determination of repeatability, accuracy and detection limits (León, et al., 2017; Soofi Abadi, et al., 2019). Determination of parasite amounts with qPCR can be performed with definite or relative quantitation. For quantification a standard curve obtained with purified parasite genomic DNA or target sequence's serial dilutions is used. With this data, target DNA found in a sample can be determined according to known DNA amount (Paiva-Cavalcanti, et al., 2010; Galluzzi, et al., 2018). In our laboratory we performed verification of the inhouse real-time PCR method with primers targeting ITS-1 gene region used for routine molecular diagnosis of leishmaniasis. With this aim, after determining the limit of detection by applying real-time PCR to 1/10 serial dilutions of DNA obtained from *L. infantum* strains growing in NNN media in our laboratory, we completed method verification studies. The results of our study showed the detection limit for our method was 0.136 copies/reaction, which is in accordance with the literature. When detection limit for target gene regions is evaluated, qPCR detection limit for kDNA and 18S primers was 1×10^{-2} parasite/ml, and qPCR detection limit for HSP70 and ITS-1 primers was reported as 1×10^{-1} parasite/ml (León, et al., 2017). Another study reported detection limit for real-time PCR method with kDNA was 0.0125 parasite DNA/ml (Mary, et al., 2004).

The obtained standard curve also provides curve used to calculate amplification efficiency. For each 10 dilutions of the target, a slope of-3.32 represents 100% efficiency and shows the amplified molecule numbers increase two times with each PCR cycle (Paiva-Cavalcanti M, et al., 2010 While slopes that are more negative than-3.32 (e.g.,-3.9) show efficiency lower than 100%, slopes more positive than -3.32 (e.g., -2.5) show problems with sample quality or pipetting (ABI_Guide_Relative_ Quantification_using_realtime_PCR). A variety of studies reported standard curves from-3.23 to-3.40

(Mary, et al., 2004; Soofi Abadi, et al., 2019). The slope of curve obtained in our study was determined as-4.097. This situation shows efficiency is lower than 100%; however, it is acceptable and indicates our DNA quality was good and there were no pipetting errors when preparing dilutions. The correlation coefficient appears to be between 0.996 to 0.998 in a various studies (Mary, et al., 2004; Hossain, et al., 2017; Soofi Abadi, et al., 2019). Similarly, correlation coefficient obtained at the end of our study was determined as 0.99.

Conclusion

In our study variation coefficient for inhouse real-time PCR method was below 15%, efficiency of the method was determined to be sufficient and decision was made that it is appropriate for use for routine molecular diagnosis of Leishmania spp. in our laboratory.

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RESEARCH ARTICLE

Platelet Lymphocyte Ratio is Associated with Carotid Atherosclerosis in Hemodialysis Patients

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Abstract

Objective: We aimed to demonstrate whether there is a correlation between Platelet Lymphocyte Ratio (PLR) and carotid intima-media thickness (CIMT) which is an early marker of atherosclerosis in patients receiving dialysis where there is a chronic inflammatory process in the body.

Methods: 53 patients receiving dialysis 3 days a week and 54 persons as the control group were included. The exclusion criteria were determined as infection, using drugs that increase or decrease the number of leukocytes such as steroids, antithyroid, chronic liver diseases, rheumatic disease, maling disease and prior cardiovascular or cerebrovascular diseases. Patients' age, gender, body mass index, hemogram and biochemical parameters were recorded. CIMT values were measured by Carotid Doppler examination.

Results: When the two groups were compared; there was no significant difference between them in terms of age, gender, blood pressures, DM, ejection fractions, lipid levels. PLR, neutrophil-to-lymphocyte ratio (NLR) and CIMT were significantly higher ($p=0.009$, $p<0.001$, $p<0.001$; respectively) in the dialysis patients group. A positive correlation was found between CIMT and PLR, NLR ($r=0.59$ $p<0.001$, $r=0.38$, $p=0.004$). As a result of the linear regression analysis, PLR (B:0.714, 95%CI:3.425/14.393, $p=0.002$) and NLR were found as independent predictors in showing CIMT.

Conclusion: PLR is independently associated with subclinical atherosclerosis assessed by CIMT in people with dialysis patients

Key words: Platelet Lymphocyte Ratio is Associated with Carotid Atherosclerosis in Hemodialysis Patients

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Introduction

The prevalence of chronic kidney disease (CKD) in the world is constantly increasing. This increase is caused by the increasing number of obese, hypertensive and diabetic patients (Wróbel et al., 2016). CKD patients are at a high risk for the development of cardiovascular disease. Cardiovascular events are an important cause of morbidity and mortality in patients with end stage renal disease (ESRD). The risk of mortality from cardiovascular disease is 30 times higher in CKD

patients compared to normal population (Yilmaz et al., 2017; Yaprak et al., 2016; Bal et al., 2015).

Atherosclerosis and chronic inflammation are a component defined in malnutrition-inflammation-atherosclerosis syndrome in ESRD patients. This syndrome is associated with negative cardiovascular events in CKD patients (Yaprak et al., 2016; Bal et al., 2015) Atherosclerosis is not only a simple vascular damage occurring with lipid infiltration, but rather is an active event with inflammatory process also involved (Horne et al., 2005). Inflammation has an important role in both beginning, and development and progression of atherosclerosis (Corriere et al., 2018; Uçar et al., 2016). Chronic inflammation which is involved in the pathogenesis of atherosclerosis is more commonly seen in ESRD patients compared to population (Ikeda 2003).

Recent studies have demonstrated that PLR shows severity of coronary artery disease, predicted major cardiovascular events and mortality, and is a significant independent predictor of major cardiovascular events. Again, studies have reported that PLR is a new inflammatory marker (Wang et al., 2017; Uzun et al., 2017; Sambel et al., 2017).

CIMT is a well-defined early marker of atherosclerosis, and has a strong correlation with the severity of coronary atherosclerosis and the risk factors of cardiovascular disease (Corriere et al., 2016) CIMT measurement is a practical and easy to apply method (Yurtdaş et al., 2014). Briefly, CIMT is a marker of subclinical atherosclerosis, and is used in prediction of cardiovascular events (O'Leary et al., 1999).

In this study, we aimed to the relationship between PLR and subclinical atherosclerosis in Hemodialysis Patients.

Methods

Patients between the ages of 18-70 were taken into the study. Our study population consisted of 53 patients with end stage renal desease who dialyzed 3 days a week and 54 control patients who visited cardiology clinic. The exclusion criteria were determined as infection, using drugs that increase or decrease the number of leukocytes such as steroids, antithyroid, chronic liver diseases, rheumatic disease (such as rheumatoid arthritis), maling disease, prior cardiovascular or cerebrovascular diseases were also excluded. Approval was obtained from the ethics committee of Ordu University.

The patients were rested for 15 minutes and their blood pressures were measured. The average of 3 measurements was taken. The weights and heights of the patients were measured with standard measurement techniques while they were hungry and standing. Body mass index (BMI) (kg/m2) was calculated using the formulas “weight (kg)/height (m)2 (Nimitphong et al., 2018).

Blood samples were taken from the patients after 8-12 hours of fasting. Biochemical parameters were studied from these blood samples and were measured cholorometrically using an Abbott original reagent on Abbott Architect 8000 auto analyzer. Beckman-Coulter Gen-S system device (Beckman-Coulter Inc., USA) was used for the complete blood count. NLR was calculated by the ratio of neutrophil count to lymphocyte count and PLR was calculated by the platelet count to lymphocyte count.

Carotid artery was performed with doppler device with 13-MHz probe. The brand of the device was Hitachi Preirus, Tokyo, Japan. The patients were lying on their back and right and left CCA imaging were performed.

Statistical analysis

The Kolmogorov–Smirnov normality test was used to evaluate the distribution of all quantitative data. Statistical Package for Social Sciences (SPSS) Version 22.0 (SPSS Inc., Chicago, Illinois. USA) was used for statistical analysis. Independent sample T test was used for continuous variables, and chi-square test for categorical variables. Non-parametric statistics was used if data were not normally distributed. Correlations were assessed using Pearson test. Lineer regression analysis was used to identify the independent predictors of KIMK. Continuous variables were defined as mean ± standard deviation, and categorical variables were given as percentages. Any P value <0.05 was considered as statistically significant.

Results

107 patients were included in the study. Control group (n=54) had normal renal function and mean age was 53,07±14,47. Patient group (n=53) had end-stage renal disease (dialyzed patients) and mean age was 54,33±7,32. 45% of the dialysis patient group and 53% of the controls were male. The clinical characteristics and the hematological and biochemical parameters of both patient groups are presented in Table 1. When the two groups were compared; there was no significant difference between them in terms of age, gender, systolic and diastolic blood pressures, presence of DM, ejection fractions, cholesterol and triglycerides levels. PLR (Figure 1), NLR and CIMT (Figure 2) were

significantly higher (p=0.009, p<0.001, p=0.003; respectively), and hemoglobin and albumin values were significantly lower (p<0.001, p<0.001; respectively) in the dialysis patient group (Table 1).

A positive correlation was found between CIMT and PLR, NLR (r=0.59 p<0.001, r=0.38, p=0.004).

As a result of the linear regression we performed to determine the independent predictors showing CIMT, PLR (Beta [B]: 0,714, 95% confidence interval [CI]: 3,425 to 14,39, P=0.002) and NLR (B: -1,218, 95%CI: -0.898 to -0.411, P<0.001) were found as the independent predictors (Table 2).

Table 1. Comparison of Dialysis Patient Group And Control Group

	In Hemodialysis Patients Group N=53	**Control Group N=54**	**P**
Age (year)	*54,33±7,32*	53,07±14,47	0,57
Gender			
Male (%)	*45*	53	0,41
BMI (kg/m^2)	*24,19±4,42*	24,80±4,10	0,45
Diabetes Mellitus (%)	*21,31*	14,03	0,34
SBP (mmHg)	*120,13±19,27*	117,83±27,12	0,60
DBP (mmHg)	*77,26±15,88*	77,51±15,19	0,90
Hemoglobin (g/dl)	*11,44±1,39*	14,12±1,94	***<0,001***
WBC (x10^3/µL)	*6,77±1,72*	6,61±1,65	0,60
PLR	*153,69±4,02*	141,43±27,68	***0,009***
NLR	*3,01±0,45*	1,87±0,36	***<0,001***
Creatinin (mg/dl)	*9,5±4,2*	0,72±0,33	***<0,001***
Total-Cholesterol (mg/dl)	*185,30±35,65*	180,2±31,20	0,43
Triglycerides (mg/dl)	*144,58±66,34*	136,62±54,6	0,50
HDL-Cholesterol (mg/dl)	*43,03±5,80*	44,8±7,12	0,14
LDL-Cholesterol (mg/dl)	*113,57±26,69*	110,55±21,8	0,55
Albumin (mg/dl)	*3,6±0,30*	4,5±,037	***<0,001***
Ejection Fraction (%)	*63,80±4,02*	64,28±3,32	0,50
CIMT	*0,73±0,23*	0,61±0,21	***0,003***
HsCRP	*3,42±1,92*	1,18±0,60	***<0,001***

BMI: Body Mass Index SBP: Systolic Blood Pressure DBP: Diastolic Blood Pressure WBC: White Blood Cell PLR: Platelet Lymphocyte Rate NLR: Neutrophil Lymphocyte Ratio CIMT: Cathodic İntima Media Thickness

Table 2. Evaluation of Independent Predictors of Carotid İntima Median

	r	**p**	**B**	**%CI**	**p**
Platelet Lymphocyte Ratio	0.59	<0.001	0,714-	3,425/14,39	0,002
Neutrophil Lymphocyte Ratio	0.38	0.004	-1,218	-0,898/-0,411	<0,001
HsCRP	0,83	<0.001	-0,828	-0,189/-0,024	0,012

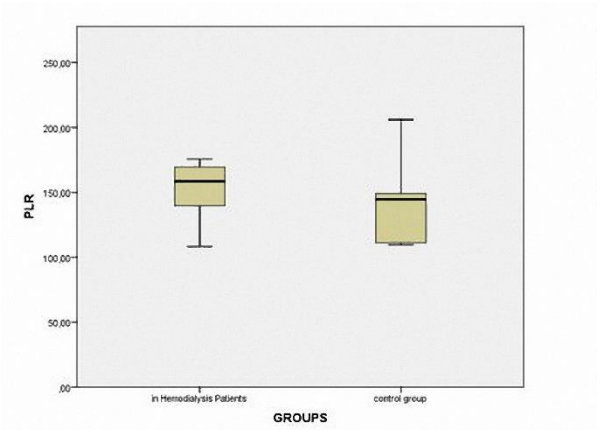

Figure 1. Comparison of mean PLR Between Control Group and in Hemodialysis Patients Group

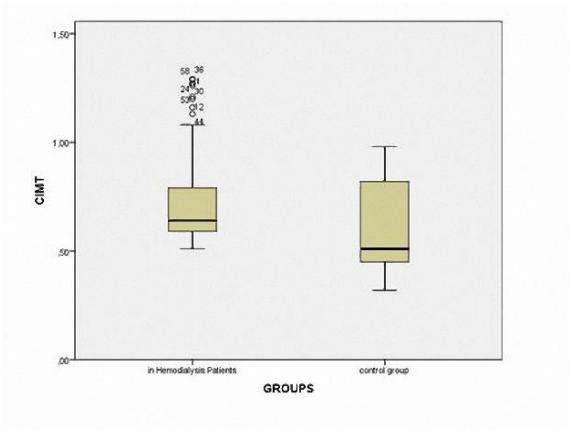

Figure 2. Comparison of mean CIMT Between Control Group and in Hemodialysis Patients Group

Discussion

In this study, we showed the relationship between PLR and CIMT as a marker of subclinical atherosclerosis in hemodialysis patients without a history of cardiovascular or cerebrovascular diseases.

Studies have reported a close association between PLR and inflammation (Samlet et al., 2017; Tola 2018; Fukuda et al., 2018). Severe and prolonged inflammation as in atherosclerosis leads to increased proliferation in megakaryocyte series, which in turn cause to an increase in platelet count (Uzun et al., 2017; Samlet et al., 2017). The bone marrow increases neutrophil count and decreases lymphocyte count during chronic stres (Yüksel et al., 2015). In addition, neutrophil count increases secondary to inflammation in cases of chronic severe continuous inflammation, and re-distribution of lymphocytes to the lymphocytic organs due to induction by stress and severe apoptosis contribute to a decrease in lymphocyte count (Corriere et al., 2018; Uzun et al., 2017).

Recent studies have investigate the place of inflammation in the pathophysiology of cardiovascular diseases, and found significant correlations between various inflammatıory markers (such as fibrinogen, C-reactive protein [CRP], interleukin-18 and tumor necrosis, factor-α, neutrophil lymphocyte ratio) and cardiovascular diseases (Ucar et al., 2016; Uzun et al., 2017; Li et al., 2017; Damman et al., 2017; Sinning et al., 2006; Celik and Bugan 2011; Kaya et al., 2014). Furthermore, studies have demonstrated a strong correlation between the number of platelets in the circulation and major cardiovascular events regardless having a known coronary artery disease (Uzun et al., 2017). Increased platelet count or increased platelet activation plays an important role in the onset and progression of atherosclerosis. (Wang et al., 2017; Uzun et al., 2017; Trakarnwijitr et al., 2017; Zhou et al., 2017). In addition, decreased lymphocyte count in patients with coronary artery disease and heart failure is reported to be associated with major negative cardiovascular events (Uzun et al., 2017; Trakarnwijitr et al., 2017; Zhou et al., 2017).

CIMT is a well-known biomarker of subclinical atherosclerosis. In addition, CIMT is a risk factor for cardiovascular disease, and a marker used in predicting cardiovascular events (Li et al., 2017; O'Leary et al., 1999).

Studies have reported a close association between the worsened prognosis and complications of cardiovascular, metabolic, malignant, and inflammatory diseases and NLR (Corriere et al., 2018; Li et al., 2017; Akbas et al., 2014; Ulu et al., 2013; Jiang et al., 2018; Polat et al., 2017; Uysal et al., 2016; Kalay et al., 2012; Xiao et al., 2014; Wei et al., 2014). In addition, NLR is stated to be a new inflammatory marker for cardiovascular risk and cardiac mortality (Uysal et al., 2016; Tatar et al., 2016). It has reported that NLR can be used as a parameter predicting mortality in end stage renal failure (Tatar et al., 2016).

Studies have reported that PLR may be an important predictor showing the prognosis of coronary artery disease (CAD) and negative cardiovascular events such as acute coronary syndrome. In addition, a high PLR level is among the independent predictors, that are used in estimating the severity of CAD (Yuksel et al., 2015; Trakarnwijitr et al., 2017; Uysal et al., 2016; Ozcan et al., 2016; Gary et al., 2013). In their study, Yuksel et al. divided the patients who underwent coronary angiography into three groups as the controls, moderate and severe groups according to the Gensini score, investigated the association with PLR, and reported that PLR predicted the severity of atherosclerosis (Yuksel et al., 2015). The roles of leukocytes and platelets in atherosclerosis are well-known. PLR which is a new prognostic marker also gives information both about aggregation and inflammation (Davi et al., 2007; Zouridakis et al., 2000). Turkmen et al. reported that PLR was a strong marker showing inflammation is ESRD patients (Turkmen et al., 2013). In their study on hemodialysis patients, Yaprak et al. found that PLR better predicted mortality than NLR.3 Compared with the other inflammatory cytokines, PLR is an easy to apply and inexpensive biomarker (Trakarnwijitr et al., 2017; Zhou et al., 2017).

As the result of the present study, PLR and NLR that are known as inflammatory markers were found significantly higher in the dialysis patient groups as expected. CIMT which is an early marker of atherosclerosis was positively correlated with PLR and NLR. The analysis showed that PLR is one of the independent predictors of CIMT.

Study limitations

Sample size was small and it was a single center design. In this study there were no data on other inflammatory markers (interleukin 10 (IL-10), interlökin 18 (IL-18), tumor necrotizing factor alpha (TNF-α) etc.)

Conclusion

In this study, increased PLR, a simple non-specific inflammatory marker, was observed to be an independent predictor of subclinical atherosclerosis assessed by CIMT in people with dialysis patients. This finding is of clinical importance, since early initiation of preventive measures may prevent the progression of atherosclerosis.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University.
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RESEARCH ARTICLE

Prevalence and distribution of colonic diverticular disease from Ordu in Turkey

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Abstract

Objective: Diverticulosis of the colon is the most frequent anatomical alteration diagnosed at colonoscopy. Most cases of diverticulosis in Western countries involve left side of the colon, while diverticulosis is predominantly present on the right side of the colon in Asian countries. With the aim of this study is to contribute to epidemiologic studies on colonic diverticula in Turkey located in Europe and Asian continent. We analyzed the data of patients undergoing colonoscopy with regard to age, gender, anatomic localization of diverticula retrospectively in the Ordu State Hospital in the Black Sea region.

Methods: We retrospectively reviewed a consecutive series of patients with regard to age, gender, location of diverticula and other colonoscopic lesions from an electronic endoscopic database who had undergone colonoscopy at the endoscopy unit of the Ordu State Hospital between 2016 and 2018. If diverticula were observed that distribution type was defined as cecum, ascending colon, transverse colon, descending colon, entire colon and sigmoid colon. In addition, we recorded that the additional diagnoses established in colonoscopic evaluations.

Results: We screened 2626 female (51.1%) and 2511 male (48.9%); totally 5137 patients from an electronic endoscopic database who had undergone colonoscopy. 227 (47.4%) female, 252 (52.6%) male, a total of 479 patients who had diverticulosis were included in this study. The mean age was determined female and male 65.44±12.01 (min 31-max 91), 64.91±10.97 (min 27-max 89) respectively and no statistically significant difference between two groups ($p>0.05$). A total of 479 patients who had diverticulosis were divided into two groups: age ≤ 50 (10.4%) and 50> 429 (89.6%). No significant difference was found with regard to diverticulosis localization between two groups ($p>0.05$). The other colonoscopic diagnoses are as follows; hemorrhoid (40.7%), polyp (40.2%), inflammatory bowel disease (4.7%) colorectal cancer (0.9%), angiodysplasia (2.3%), ulcer (0.9%), anal fissure (1.9%), parasitosis (1.9%), terminal ileitis (0.5%), nonspecific colitis (3.7%), bowel surgery (1.9%) and submucosal lesion (0.5%).

Conclusion: Diverticular disease is a worldwide condition that affect elderly people with an increasing incidence in younger patients as well as in developing countries that have started adopting Western diets. So that in the future diverticular disease will induce a significant economic burden in terms of healthcare cost in Turkey. Despite its prevalence, its pathophysiology still remains poorly understood. There is a significant need for more studies to improve our understanding about diverticular disease.

Key words: Colonoscopy, colonic diverticular disease, diverticulosis localization.

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Introduction

The presence of the diverticula in the colon is known as diverticulosis. Diverticulosis is asymptomatic frequently in some patients. However, it can be symptomatic and complicated clinical course (Tursi et al., 2015).

For many years it has been assumed that most cases of diverticulosis were in the Western hemisphere and was due to the absence of fiber intake in the diet (Floch and Bina, 2004), nevertheless, recent studies show that the prevalence of diverticulosis has increased all over the World over the years (Alatise et al., 2012). The prevalence rates of diverticulosis were reported in Western and Asian countries 5-45%, 13-25%, respectively. In Western nations, diverticula primarily involve left-sided in contrast with Asian population, anatomic distribution is predominantly right-sided (Parks, 1969; Ngoi et al., 1992). Diverticulosis is often seen in a population of 70 years old (Painter and Burkitt, 1975). Diverticular disease clinically has been classified into four types; symptomatic complicated by diverticulitis, diverticular bleeding, segmental colitis and symptomatic uncomplicated diverticulitis (Imaeda and Hibi, 2018). Diverticular disease is the cause of a very high gastrointestinal symptom-related hospital admission. Its incidence has been shown to increase over time in both Western and Asian population (Nagata et al., 2014). This increase in the incidence of the disease will become a major of economic burden in terms of healthcare-costs (Imaeda and Hibi, 2018).

In this study, we intended to determine the prevalence and distribution of colonic diverticular disease from Ordu in Turkey.

Methods

Design of Study: This study was a retrospective and single-centre study. We analyzed the database of 5360 patients undergoing colonoscopy from December 2016 to January 2019 in Ordu State Hospital. Indication for colonoscopy were documented as follow; patients with lower GI complaints, asymptomatic patients with anemia; to screen patients with colorectal cancer in their family history, patients who required to screen for the positive faecal occult blood test, patients who underwent therapeutic endoscopy. We did not include patients with less than 18 years, those whose colonoscopic evaluation was restricted to the rectum, and patients with poorly stool cleaning. We excluded 223 patients who had one or more of the exclusion criteria. Finally, 5137 patients were selected for analysis and 449 patients had colonic diverticula. Diverticula was recorded in electronic endoscopy report according to anatomical localization. Distribution type was defined as the cecum, ascending colon, transverse colon, descending colon, entire colon and sigmoid colon. The information of patients were accessed from an electronic endoscopic database and archive files. The sample size was calculated with the incidence of diverticular disease (10%) and population size (The population of Ordu centre is 750000) and was determined to be 4098 people in 95% confidence interval.

The patients were recommended clear liquid diet two days ago that were given sennoside a + b calcium 500 mg/250 mL (X-M solution®-Yenisehir Laboratory, Turkey) orally and sodium dihydrogen phosphate + disodium hydrogen phosphate (B.T. Enema® 210 mL-Yenisehir Laboratory, Turkey) rectally one day ago colonoscopy. The procedures were performed under general anesthesia with intravenous administration of 0,05 mg/kg midazolam (Midolam, Pharmada pharmacy, Turkey). Colonoscopic evaluation were performed with XQ Olympus (Olympus, Corporation, Tokyo, Japan) endoscopy device by gastroenterology specialist.

This study was approved by the institutional review Clinical Research Ethics Committee of Ordu University.

Statistical analysis:

All statistical analyses were performed with Statistical Package for Social Science (SPSS) software (IBM SPSS version 24.0 for Windows, Chicago, USA). P value less than 0.05 was considered as statistically significant. Descriptive statistics for continuous variables; mean, standard deviation (SD), minimum and maximum values; expressed as number and percentage for categorical variables. The Mann-Whitney U test was performed for continuous variables. The Pearson's chi-square test was used to determine the correlation between the categorical variables.

Results

We screened 2626 female (51.1%) and 2511 male (48.9%); totally 5137 patients from electronic endoscopic reports. Among 227 (47.4%) female, 252 (52.6%) male, a total of 479 patients who had diverticulosis were included in this study. In terms of gender, detection rate of diverticulosis on colonoscopy that was found female and male; 8.6% (n: 227/2699), 10% (n: 252/2511), respectively.

The mean age was determined female and male respectively 65.44±12.01 (min 31-max 91), 64.91±10.97 (min 27-max 89) was no statistically significant difference between in two groups (p>0.05). Table 1 shows the distribution of diverticula on localization.

There was no statistically significant difference between age and localization. (p>0.05). The incidence of diverticular disease in all patients evaluated under 50 years and older. A total of 479 patients who had diverticulosis were divided into two groups: age ≤ 50 (10.4%) and age>429 (89.6%). No significant difference was found with regard to diverticulosis localization between two groups (p>0.05). Distribution of diverticula on gender and age groups are given in table 2.

Other colonoscopic diagnoses were determined in 214 (44,7%) patients. These diagnoses were shown in table 3.

Table 1. The Distribution of Diverticula by Localization

Localization	**N**	**%**
Cecum	67	14
Ascending colon	118	24,6
Transverse colon	125	26,1
Descending colon	324	67,6
Sigmoid colon	379	79,1
Entire colon	46	9,6
Total	1059	

N: Number, %: Percent.

Table 2. Distribution of Diverticula on Gender and Age Group

Localization	Gender		*P value**	Age		*P value**
	Female (n=227)	Male (n=252)		≤50 Age, % (n=50)	>50 Age, % (n=429)	
Cecum	33 (49,3%)	34 (50,7%)	0,74	10 (14,9%)	57 (85,1%)	0,20
Ascending colon	57 (48,3%)	61 (51,7%)	0,82	11 (9,3%)	107 (90,7%)	0,65
Transverse colon	59 (47,2%)	66 (52,8%)	0,96	10 (8%)	115 (92%)	0,30
Descending colon	153 (47,2%)	171 (52,8%)	0,92	23 (7,1%)	301 (92,9%)	0,01
Sigmoid colon	172 (45,4%)	207 (54,6%)	0,87	31 (8,2%)	348 (91,8%)	0,02
Entire colon	21 (45,7%)	25 (54,3%)	0,80	4 (8,7%)	42 (91,3%)	0,68

*: Chi-square test was used. P value of <0.05 was considered statistically significant

Table 3. Distribution of the Additional Diagnoses Established in Colonoscopic Examinations.

Diagnosis	N	%
Hemorrhoids	87	40,7
Polyp	86	40,2
Inflammatory bowel disease	10	4,7
Colorectal cancer	2	0,9
Angiodysplasia	5	2,3
Ulcer	2	0,9
Anal fissure	4	1,9
Parasitosis	4	1,9
Terminal ileitis	1	0,5
Nonspecific colitis	8	3,7
Bowel surgery	4	1,9
Submucosal lesion	1	0,5
Total	214	

N: Number, %: Percent.

Discussion

Diverticulosis of the colon is an anatomical change manifested by pouches in the wall of the colon. Diverticula occurs due to herniated mucosa and submucosa through the defect in the muscular layer of the colon. Impairment colonic motility is the most important preparatory factor in the development of diverticula. The pathophysiology of diverticula includes age, genetic factors, changing colonic motility, lifestyle conditions such as smoking, obesity, alcohol consumption, physical inactivity, fiber and meat intake with diet (Alessandra et al., 2018).

Recent research has indicated a rising in the prevalence of the diverticular disease in the world (Alatise et al., 2012). According to some studies conducted at the beginning of the 20th century; a diverticular disease incidence between 2-10% and 5-20% in patients in developing countries (Painter and Burkitt, 1971). Distribution of diverticular disease by gender tends to change over the years. Previously the diverticular disease was the most common in males. In addition, new studies represent that males under 50 years old have a higher incidence of disease. A study conducted in the United Kingdom showed that the disease is more often in females after the 4th decade (Warner et al., 2007). In some countries prevalence of the disease can be listed as follows: In Western countries 5-45%, in Asia 13-25%, in Lebanon 33%, in the Far East 13-25%, Korea 12 % and Taiwan 14% (Painter and Burkitt, 1971, Song et al., 2010; Sharara et al., 2013; Wang et al., 2015).

The diverticular disease is rare in sub-Saharan countries (Baako, 2001). Nigeria declared an incidence as low as 9.4% among patients that undergoing colonoscopic examination (Alatise et al., 2012). The incidence of diverticular disease is low in African countries too. Because African people have limited access to health care (Blachut et al., 2004).

Celebi et al. (2017) divided all patients into two groups as the age of 15-64 years and age of ≥65 years. The incidence of diverticula was higher in the group over 65 year of age than age of 15-64 years (12.7% vs. 4.5%). However, no statistical difference was found between female and male patients at single center study in Turkey. Additionally, in this study, the frequency of diverticular disease was higher in the patients 50 ≥ years old than in those under the age of ≤50 years old. We also found no difference in the incidence of diverticular disease between female and male patients.

Celebi et al. (2017) presented that %6.8 rate of diverticulosis in patients undergoing colonoscopy. Tamer et al. (2005) reported that diagnoses and rates as follows; hemorrhoid, polyps, ulcerative colitis, diverticula and colorectal cancer; 33.4%, 14.1%, 4.7%, 4.1% and 3.7%, respectively (12). Yilmaz et al. (2006) analyzed retrospectively the data of 322 patients who underwent colonoscopy. They explained that the disease rates as follows: hemorrhoid (17.7%), polyps (14.9%), ulcer (12.4%) and masses (12.4%) and did not report rate of diverticular disease. In our study, 40.7% hemorrhoid and diverticulosis 9.3% were detected

and these rates are higher than Tamer et al. (2005) and Celebi et al. (2017). The total number of patients in the four different studies as follows; 2069 (Tamer et al., 2005), 322 (Yilmaz et al., 2006), 2831 (Celebi et al., 2017), and 5137 (our study).

Anatomical localization of the diverticula varies by race and geography. Diverticula are located only sigmoid colon in 65% of patients, in 24% of patients mainly located in the sigmoid colon, and in %7 of patients' diverticula involve entire colon in the Western and industrialized population (Parks, 1969). The Asian population, diverticula principally involves the right colon with a rate of approximately %13 to %25 (Ngoi et al., 1992). In our population, distribution of diverticula; cecum 14%, ascending colon 24.6%, transverse colon 21.65%, descending colon 67.6%, sigmoid 79.1%, entire colon 9.6% that reflected both Western and Asian population.

With the increasing incidence of diverticular disease continues to be a burden in terms of health care costs in Western and Asian countries (Imaeda and Hibi, 2018). According to our predict that the incidence of diverticular disease will increase because of rising elderly population over the years in Turkey. At the same time, the transition of a Western type lifestyle lead to an increase in diverticulosis incidence in youth patients. There are two important complications in diverticular disease: diverticulitis and diverticular bleeding. Diverticular bleeding occurs 5 to 15 percent among all patients. In 4 to 15 percent of patients with diverticular disease may develop diverticulitis (Imbembo and Bailey, 1992).

About 200000 patients have been admitted to the hospital each year due to symptoms related to diverticular disease in the United States (Peery et al., 2015). Delvaux (2003) reported that there were almost 800000 hospital admissions for diverticular disease in European countries. So that in the future diverticular disease will be a significant healthcare problem in Turkey. We need to know the true prevalence to reduce mortality and morbidity associated with diverticular disease. The number of large-scale studies should be increasing all over the world. Prevalence of diverticulosis has been estimated prospectively in several colonoscopy-based studies in Asia. We can show the biggest deficiencies in our study as follows: unrecorded patient symptoms in the evaluation and the absence of out-hospital follow-up of the patients and this study was retrospective. We think that there is a need for multicentric epidemiological studies on this subject in Turkey.

Conclusion

Diverticular disease affects especially the elderly population in worldwide. However, new studies have begun to show an increased incidence in the young population. The most important reason for this is the developing countries that have started transition Western diets. Despite the rapid increase in the prevalence of the disease, its pathophysiology remains unresolved. Diverticular disease should be considered as a major burden of health-care cost for all over the world. There is a need for new studies to reduce the prevalence of diverticular disease.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Ordu University.
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RESEARCH ARTICLE

The Knowledge and Experience of Nursing Students about Occupational Health and Safety

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Abstract

Objective: This study was conducted to find out the knowledge and experience of student nurses on occupational health and occupational safety.

Methods: This descriptive study was conducted on nursing students studying in the Health Sciences Faculty of a University between 26 May and 16 June 2017. The universe of the study consisted of all students studying in the department of nursing (N=446), while the sample consisted of 332 student nurses determined according to "Raosoft" sample size calculation program. The data were collected through face to face interview technique with "Questionnaire Form" developed by the researchers.

Results: It was found that 76.5% of the student nurses in the study were trained about occupational health and safety before they went out to practice areas and 85.5% were vaccinated before they went out to clinical areas. 43.7% of the student nurses stated that they had occupational accident, 31% of these stated that they were injured with a sharp object and 67.6% stated that they did not report the accident. A statistically significant association was found between student nurses' ages, their year of study and their state of experiencing occupational accident ($p<0.05$).

Conclusion: It was found that almost half of the student nurses in the study underwent occupational accident, most of the reasons for occupational accidents was sharp object injury and most of the students who had occupational accident did not report these.

Key words: Student nurse, Occupation, Health, Safety, Accident

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Introduction

Occupational health and safety includes the works to maintain and increase physical, mental and social well-being of employees in all occupations (Bilir and Yildiz, 2012). Occupational health and safety is made up of two different concepts coming together. Occupational health is related with working conditions. It means the elimination of dangerous situations that can occur from equipment used in the working environment. Occupational safety includes the necessary technical, medical and legal precautions to be taken for employees not to experience physical and mental losses in the work place (Bayilmis, 2013). Each year, 2.3 million

people are exposed to work-related accidents or diseases in the world and more than 6000 people lose their lives every day because of occupational accidents. It is reported that globally 340 million occupational accidents occur and 160 million people die due to occupational accidents each year. However, it is reported that there aren't enough notices about occupational accidents (International Labour Organization, 2018).

Since health sector is one of the most risky lines of work, occupational accidents and diseases that occur in health institutions can cause serious consequences (Akgun, 2015). People working in health sector are faced with the risk of occupational diseases, occupational accidents, injuries and death every day in their work places through the effects of biological, chemical, physical, ergonomic and psycho-social factors, mainly contagious diseases (Sagoe-Moses et al., 2005; Bilir and Yildiz, 2012). In terms of occupational health and safety, nursing is reported to be a high risk occupation globally (Aiken et al., 2002). Nurses can be exposed to occupational accidents and risk factors, mainly infectious diseases, chemical agents, physical agents and sharp object injuries not only in their occupational lives, but also during their educational life due to their limited knowledge and experience (Shiao et al., 2002; Smith and Leggat, 2005; Merlin et al., 2011; Isara and Ofili, 2012; Lauer et al., 2014; Savcı et al., 2018). In some studies, it is stated that 28.8% to 69.8% of the students were exposed to occupational accidents during their education (Talas 2009; Togan et al., 2015; Karatas et al., 2016; Celikkalp et al., 2017; Savcı et al., 2018).

In Turkey, with the law of Occupational Health and Safety and the regulations issued in line with this law, all health institutions, including hospitals, and health professionals and students working in these institutions were included into the scope of social security and it has become compulsory to apply the regulations related with occupational health and security (Babayigit, 2014). Within this scope, it is important to assess the knowledge and practices of student nurses about isolation precautions, their states of experiencing occupational accident and their knowledge and experiences about occupational health and safety and thus to determine factors about students and institutions in terms of occupational health and safety precautions. This study was conducted to find out student nurses' knowledge and experience about occupational health and safety.

Methods

Study design population and sampling

This descriptive study was conducted on nursing students studying in the Health Sciences Faculty of a University between 26 May and 16 June 2017. The universe of the study consisted of all students studying in the department of nursing at Health Sciences Faculty (N=446). The sample was chosen from the universe through "Raosoft" sample size calculation program (http://www.raosoft.com/samplesize.html). Although the calculation showed that there had to be 207 samples within 95% confidence interval, the study was completed with 332 students to get closer to the actual data of the population. Nursing students who actively continue their education and willing to participate were included in the study.

Ethical considerations

In terms of ethics, ethical consent was taken from Ordu University Clinical Researches Ethical Board (Decision no: 2017/68), written permission was taken from the deanship of the Faculty of Health Sciences and verbal consent was taken from the students who participated in the study.

Data Collection Instruments

The data were collected through "Questionnaire Form" developed by the researchers in line with the literature (Smith and Leggat, 2005; Talas, 2009; Karatas et al., 2016; Celikkalp et al., 2017; Savci et al., 2018). The Questionnaire Form consists of 4 parts. The first part includes 8 questions about student nurses' identifying characteristics (age, gender, year of study, having been trained about occupational health and safety, the trainer, having been trained about vaccination, reasons for getting or not getting vaccinated). The second part includes 11 questions about student nurses' knowledge and practices about isolation precautions (knowing under which circumstances personal protective equipment is to be used, being informed by nurses about the patient who has isolation precautions, making use of personal protective equipment, knowing what contact isolation is, knowing what droplet isolation is and its sign, making interventions on patients with droplet isolation, using personal protective equipment while making interventions on patients with droplet isolation, knowing about respiratory isolation and its sign, encounter with a patient with respiratory isolation, using personal protective equipment while making interventions on patients with respiratory isolation).

The third part includes 4 questions about student nurses' characteristics related with occupational safety (thinking that the workplace has enough warning, being warned enough by instructors about occupational safety, being sensitive about occupational safety rules, being willing about receiving a course on occupational health and safety in undergraduate education). The fourth part includes 5 questions about student nurses' occupational accident characteristics (having experienced occupational accident, areas in which they experienced occupational accident, situations in which they experienced occupational accident, whether the occupational accident was reported, and the reasons for not reporting the occupational accident).

Data Collection

The data were collected by the researchers in the classroom between 15 to 20 minutes by using face to face interview technique.

Data Assessment

The data were assessed by using SPSS 20.0 program. Descriptive statistics (numbers, percentage, arithmetic mean, and standard deviation), Independent –Samples T-Test and Chi-square test were used to assess the data. Descriptive statistics were used to determine socio-demographic characteristics, isolation measures, occupational safety and occupational accident characteristics of the students. In addition, t-test and chi-square test were used to compare students' socio-demographic characteristics and occupational accident situations. Level of significance was 0.05.

Results

Average age of the nurses in the study was 20.89±1.85 (Min:18; Max: 28), 72% were female and 30.7% were in their first year of study. It was found that 76.5% of the student nurses had been trained about occupational health and safety before they went out to practice areas and 66.3% were found to be trained by occupational health and safety expert. 73.2% of the student nurses stated that they were trained before they went out to practice areas, 34.3% stated that they were vaccinated before they went out to practice areas and 51.2% stated that they did not get vaccinated because they were immune (Table 1).

It was found that 90.7% of the student nurses knew what isolation precautions are, 41.9% were informed by the nurses about patients who had isolation precaution in practice areas, 58.7% could make use of personal protective equipment in practice areas, 32.8% used Surgical mask/N 95 mask while making interventions on patients with droplet isolation and 43% used Surgical mask/N 95 mask while making interventions on patients with respiratory isolation (Table 2).

It was found that 56.9% of the student nurses thought there were enough warnings about occupational safety in practice areas, 62.7% were warned enough by instructors about occupational safety and 65.1% were willing to receive course on occupational health and safety in undergraduate education (Table 3).

It was found that 43.7% of the student nurses experienced occupational accident and 47.5% of these had experienced occupational accident in the clinic. Of the students who experienced occupational accident, 31% had accident with a sharp object, 13.6% with a clean instrument before treatment, 13.3% while getting drug from the ampoule, 9.6% while closing the tip of the needle and while separating the needle from the injector during treatment, 9% with an instrument which had blood on, 5.1% while diluting the drugs, 4.5% during IV practices and while bloodletting, 4.2% while throwing wastes in the waste box and while getting drug from the vial, 3% while cleaning supplies, 2.4% while giving intravenous/intramuscular drug, 2.1% due to transmission to the eye (blood, body fluid, drug, etc), 1.5% with an instrument in a friend's hand, 1.2% while giving stitches, 0.9% while transferring blood to tube and while putting on cannula. 67.6% of the student nurses stated that they didn't report the occupational accident and when the reasons for not reporting were examined, it was found that 27.5% did not report because they didn't know that they had to report, 22.4% because there was no risk of being infected by the patient, 19.3% because they did not know about the reporting process, 33.7% because they were injured with a sterile instrument, 20.4% because they were not anxious, 9.1% because they did not have time and 5.1% because they were immune (Table 4).

A statistically significant association was found between student nurses' ages, their year of study and their state of having experienced occupational accident ($p<0.05$) and as age and year of study increased, it was found that they experienced more occupational accidents (Table 5).

Table 1. Distribution of identifying characteristics of student nurses

Identifying Characteristics		N	%
Average age		20.89±1.85 (Min:18; Max:28)	
Gender	Female	239	72.0
	Male	93	28.0
Year of study	1st year	102	30.7
	2nd year	50	15.1
	3rd year	79	23.8
	4th year	101	30.4
Having been trained about occupational health and safety before going out to practice areas	Yes	254	76.5
	No	78	23.5
Trainer	Instructor of the course	32	9.6
	Occupational health and safety expert	220	66.3
	Chief nurse	10	3.0
	Infection nurse	5	1.5
	Others	4	1.2
Having been trained about vaccination before going out to practice areas	Yes	243	73.2
	No	89	26.8
Having been vaccinated before going out to practice areas	Yes	114	34.3
	No	218	65.7
Reasons for not getting vaccinated	I was immune	170	51.2
	I didn't know I had to	17	5.1
	I didn't care	16	4.8
	Other	16	4.8

Table 2. Distribution of student nurses' knowledge and practices about isolation precautions

Knowledge and Practices about Isolation Precautions		N	%
Knowing what isolation precautions are	Yes	301	90.7
	No	31	9.3
Knowing under which circumstances personal protective equipment will be used	Yes	291	87.7
	No	41	12.3
Being informed by the nurses about patients who have isolation precaution in the practice area	Yes	139	41.9
	No	68	20.5
	Sometimes	125	37.7
Making use of personal protective equipment in practice areas	Yes	195	58.7
	No	43	13.0
	Sometimes	94	28.3
Knowing what contact isolation is	Yes	294	88.6
	No	38	11.4
Knowing about droplet isolation and its sign	Yes	294	88.6
	No	38	11.4
Making intervention on patients with droplet isolation	Yes	105	31.6
	No	227	68.4
Using personal protective equipment while making intervention on patients with droplet isolation	Gloves	102	30.7
	Surgical mask/N 95 Mask	109	32.8
	Apron	26	7.8
	Glasses	6	1.8
Knowing about respiratory isolation and its sign	Yes	287	86.4
	No	45	13.6
Encounter with patients with respiratory isolation	Yes	144	43.4
	No	188	56.6
Using personal protective equipment while making intervention on patients with respiratory isolation	Gloves	101	30.4
	Surgical mask/N 95 Mask	143	43.0
	Apron	26	7.8
	Glasses	6	1.8

Table 3. Distribution of student nurses' characteristics related with occupational safety

		N	%
Thinking that there are enough warnings about occupational safety in practice areas	Yes	189	56.9
	No	143	43.1
Being warned enough by instructors about occupational safety	Yes	208	62.7
	No	124	37.3
Being sensitive about occupational safety	Yes	202	60.8
	No	130	39.2
Being willing about receiving a course on occupational health and safety in undergraduate education	Yes	216	65.1
	No	116	34.9

Table 4. Distribution of student nurses' characteristics related with occupational accident

		N	%
Having experienced occupational accident	Yes	145	43.7
	No	187	56.3
Areas in which occupational accidents were experienced	Clinical	69	47.5
	Bloodletting	28	19.3
	Intensive care	11	7.5
	Emergency service	16	11.0
	Other areas	21	14.7
Situations in which occupational accidents were experienced	Injury with a sharp object	103	31.0
	Injury with a clean instrument before treatment	45	13.6
	Injury with an instrument which has blood on	30	9.0
	Injury while closing the tip of the needle during treatment	32	9.6
	Injury while separating the needle from the injector	32	9.6
	Injury while stitching	4	1.2
	Injury while throwing the waste in waste box	14	4.2
	Injury with an instrument in a friend's hand	5	1.5
	Injury during IV practices	15	4.5
	Injury while cleaning supplies	10	3.0
	Injury while transferring the blood to the tube	3	0.9
	Injury while getting drug from the ampoule	44	13.3
	Injury while getting drug from the vial	14	4.2
	Injury while diluting the drug	17	5.1
	Injury while giving intravenous/intramuscular drug	8	2.4
	Injury while putting on IV cannula	3	0.9
	Injury while bloodletting	15	4.5
	Injury with transmission to the eye (blood, body fluid, drug, etc)	7	2.1
	Other	4	1.2
Having reported the occupational accident	Yes	47	32.4
	No	98	67.6
Reasons why occupational accidents were not reported	I didn't know that I had to report	27	27.5
	Since there was no risk of infection from the patient	22	22.4
	I didn't know about the reporting process	19	19.3
	Since I was injured with a sterile instrument	33	33.7
	I wasn't worried	20	20.4
	I didn't have time	9	9.1
	I was immune	5	5.1

*More than one answer was given to situations in which occupational accidents were experienced and the reasons for not reporting the occupational accident.

Table 5. Comparison of student nurses' states of occupational accident experience and their identifying characteristics

Average age			Occupational Accident Experience	
			Yes	No
		X±SD	21.17±1.64	20.66±1.97
	Test/p value		t=2.507 p=0.013	
Gender	Female	n	99	140
		%	41.4	58.6
	Male	n	46	47
		%	49.5	50.5
	Test/p value		X^2=1.759 p=0.185	
Year of study	1st year of study	n	33	69
		%	32.4	67.6
	2nd year of study	n	17	33
		%	34.0	66.0
	3rd year of study	n	41	38
		%	51.9	48.1
	4th year of study	n	54	47
		%	53.5	46.5
	Test/p value		X^2=13.325 p=0.004	
Having been trained in occupational health and safety before going out to practice areas	Yes	n	112	142
		%	44.1	55.9
	No	n	33	45
		%	42.3	57.7
	Test/p value		X^2=0.077 p=0.781	

t=Independent –samples t-test X^2=Chi-square test

Discussion

The findings of this study, which was conducted to find out student nurses' knowledge and experience about occupational health and safety, were discussed in line with the literature. In the study, it was found that 76.5% of the student nurses had been trained about occupational health and safety before they went out to practice areas and 66.3% had been trained by an occupational health and safety expert. In a study conducted by Talas, it was found that 84.8% of nursing students were trained about occupational health and safety, while it was found by Savcı et al. that 90.5% of students studying in disciplines about health were trained about occupational health and safety (Talas, 2009; Savcı et al., 2018). In addition, Togan et al. found that 22.6% of the students studying at vocational health schools were trained about occupational accidents (Togan et al., 2015). Our results are in parallel with the results in literature. It is thought that this rate was high in our study due to the fact that it is obligatory for all students studying in the field of health to be trained about occupational health and safety before beginning to work in clinical areas with the latest regulations in Turkey. In our study, it was found that 73.2% of the student nurses had been trained about getting vaccinated before going out to practice areas, 34.3% had been vaccinated and of the students who had not been vaccinated, 51.2% had not been vaccinated since they were immune and 85.5% had gotten vaccinated before going out to clinical areas. Similarly, in studies conducted on student nurses, it was found that 67.7% and 73.5% and 83.6% of the students in vocational health schools had been vaccinated for Hepatitis B (Talas, 2009; Togan et al., 2015; Karatas et al., 2016). It is thought that the high rate of being vaccinated in our study can be due to the fact that students are trained about getting vaccinated before going out to clinical areas.

In our study, it was found that the knowledge level of student nurses about isolation precautions was higher than 80%; however, the rates of making use of personal protective equipment to apply isolation precautions was found to be low. Students in the field of health should know under which circumstances isolation is applied and which isolation includes what (Tayran and Ulupınar, 2011). In a study conducted by Barikani and Afaghi on Irish medical students, it was found that students had high levels of knowledge about standard isolation precautions, but they were not sufficient in terms of practice (Barikani and Afaghi, 2012). In studies conducted by Alotaibi et al. and Suliman et al. it was found that students had high level of knowledge about isolation precautions; however, organizational factors and work load influenced nurses' isolation practices and nurses had to be supported on this issue (Alotaibi et al., 2017; Suliman et al., 2018). In parallel with the literature,

it was found in our study that student nurses had high level of knowledge about isolation precautions but they did not make enough use of personal protective equipment in clinics to apply isolation precautions. It is thought that the reason for this can be the fact that personal protective equipment are planned according to the number of health professionals.

In the study, it was found that 43.7% of the nurses had experienced occupational accident. Talas reported that 49% of nursing students had occupational accidents, Celikkalp et al. reported that nursing students in their third year of study had at least one occupational accident during their practical training and Karataş et al. reported that 28.8% of the students nurses had been injured with a sharp object (Talas, 2009; Karatas et al., 2016; Celikkalp et al., 2017). Togan et al. reported that 68.7% of the students in vocational health schools had been injured with a sharp object and Savcı et al. reported that 69.8% of the students in health disciplines were exposed to occupational accident at least once (Togan et al., 2015; Savci et al., 2018). The results of our study are in parallel with the results of studies conducted on nurse students. In our study, it was found that nurse students experienced occupational accidents with sharp objects, with clean instruments before treatment, while getting drug from the ampoule, while closing the tip of the needle during treatment, while separating the needle from the injector, with an instrument which had blood on, while diluting the drug, during IV practices, while bloodletting, while throwing the waste in waste box, while getting drug from the vial, while cleaning supplies, while giving intravenous/intramuscular drug, with transmission to the eye (blood, body fluid, drug, etc), with an instrument in a friend's hand, while stitching, while transferring the blood to the tube and while putting on IV cannula. In studies conducted on nursing studies in literature, it was found that students experienced occupational accidents while putting on IV catheter, using scalpel, bloodletting tubes, glass material, insulin injector, suture needles, blood sugar lancet, butterfly needle, injector, clean instrument before treatment, while closing the needle tip during drug application, while closing the needle tip after injection/treatment, while disposing of the needle to the waste box, with accidental prick of an instrument with blood on it, during venous bloodletting and while putting on IV cannula (Smith and Leggat, 2005; Talas, 2009; Canli Ozer and Aydin Bektas, 2012; Karatas et al., 2016). On the other hand, Togan et al. found that students in vocational health school experienced occupational accidents through needlestick injury, while closing the tip of injection, with injection in the trash, with blood coming to eye or an open wound and as a result of injury through surgical instrument and Savcı et al. reported that students in health disciplines experienced occupational accidents as a result of sharp object injuries, infection with contaminated bodily fluid, being exposed to x-rays and intoxication (Togan et al., 2015; Savcı et al., 2018). In parallel with the literature, student nurses in our study were found to experience occupational accidents due to sharp object injury mostly. It is thought that this may be due to the fact that nurses do not have enough laboratory practices before they go out to clinics.

It was found that 67.6% of the student nurses did not report occupational accidents and the reasons for not reporting were being unaware of the necessity to report, no risk of transmission from the patient, not knowing about the process to report, being injured with sterile instruments, not worrying, not being able to allocate time and being immune. Smith and Leggat found that 86.1% of nursing students did not report occupational accidents and the reasons for not reporting were not knowing how to report, having minor injuries and thinking that they would get in trouble (Smith and Leggat, 2005). Talas found that 43.9% of nursing students reported occupational accidents and the reason for not reporting was because the injury was minor (Talas, 2009). Karatas et al. found that 18.7% of nursing students reported occupational accidents and the reasons for not reporting were not being aware of the procedure, the disease having a low risk of contamination and not being worried (Karatas et al., 2016). In parallel with the literature, the rates of students reporting occupational accidents were low and the reasons for not reporting were similar. It is thought that although most of the student nurses had been trained about occupational health and safety in the study, the fact that reporting process was not emphasized much caused the low rates of reporting.

It was found that student nurses' ages and years of study influenced their state of experiencing occupational accidents, while gender and being trained about occupational health and safety did not. The students were found to experience more occupational accidents as their ages and years of study increased. Similarly, Smith and Leggat found that students' rates of being exposed to occupational accident increased as their years of study increased, while their ages, genders and other characteristics did not influence their rate of experiencing

occupational accidents (Smith and Leggat, 2005). On the other hand, unlike our results, Canli Özer and Aydin Bektas found that nursing students experienced the highest number of sharp object injuries in their second year of study and the lowest number in their fourth year of study and Karataş et al. found that nursing students experienced the highest number of occupational accidents in their second year of study and the lowest number in their fourth year of study and that gender did not influence the state of experiencing occupational accident (Canli Ozer and Aydin Bektas, 2012; Karatas et al., 2016). It is thought that the reason why student nurses experience more occupational accidents towards the last year of their study is the fact that they are exposed to more clinical environment and accidents towards the end of their study.

Limitations of the study

The study has some limitations. First of all, the fact that the study was conducted on nursing students of only one university is a limitation in terms of the generalization of the results. Secondly, since the study was conducted on first, second, third and fourth year students, it is possible that students may have forgotten the answers to some questions due to memory factor.

Conclusion

It was found that most of the student nurses in the study had been trained about occupational health and safety and had been vaccinated before they went out to practice areas. It was found that most of the students knew about isolation precautions; however, they did not make enough use of personal protective equipment. Almost half of the students stated that there weren't enough warnings about occupational safety in practice areas and it was found that most of them were willing to receive a course on occupational health and safety in their undergraduate education. It was found that almost half of the students had experienced occupational accident and most of these students had been injured with sharp objects and did not report the occupational accident. In line with these results, it is recommended to increase awareness about occupational accidents, reasons and reporting these starting from first year of study. It is recommended for instructors to have knowledge about occupational health and safety and to be supported by institutional managers, nurses and instructors so that they can observe students and help them to apply isolation precautions in practice areas.

Ethics Committee Approval: Ethics committee approval was received for this study from Ordu University Clinical Research Ethics Committee.
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RESEARCH ARTICLE

Does Symphysis Pubis Width Change During Labor?

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Abstract

Objectives: The anatomical and dynamic structure of the pelvis is an important element in delivery. During pregnancy, high progestorene and relaxin levels produce physiological ligament relaxation on the pelvis. The suitability of pelvic diameters is important in performing vaginal delivery. In this study we aimed to determine whether there is any change in symphysis pubis width during delivery.

Methods: This prospective observational study included 108 pregnant women who were at 37-42 weeks of gestation with cephalic presentation of a singleton pregnancy and were hospitalized for delivery. Symphysis pubis width was measured by transabdominal ultrasonography in each woman during the latent phase while cervical dilatation was 2 cm, the active phase while cervical dilatation was 6 cm and the second stage within one hour of complete cervical dilatation.

Results: Symphysis pubis width value significantly increased as the labor progressed. Namely, the mean symphysis pubis width value measured in the latent phase was the smallest while the mean value in the second stage of the labor was the highest. In addition, birth weight of newborn was positively correlated each of symphysis pubis width values measured at all three different times.

Conclusion: Symphysis pubis width increases as labor progresses. And this increase advances as the weight of newborn increases.

Key words: Symphysis pubis; ultrasonography; labor

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Introduction

The anatomical and dynamic structure of the pelvis is an important element in delivery. Therefore, in obstetric practice, during vaginal delivery, it is always evaluated in optimal conditions for a safe and successful delivery (Gruss and Schmitt, 2015)

The joints between the bones that make up the pelvis are hard and strong joints and have minimal mobility. This allows the pelvis to form a solid structure and fully support the human skeleton (Lewis et al., 2017). Furthermore, the suitability of pelvic diameters is important in performing vaginal delivery (Maharaj, 2010). Namely, a small

expansion of pelvic diameters is needed for the safe progression of vaginal delivery. Therefore, there may be some changes in the structure of pelvis during pregnancy. Changes in pregnancy and especially with hormonal effects, softening and loosening of the symphysis pubis (SP) and sacroiliac joints positively contribute to the progression of labor. This adaptation is characteristic for the harmony between fetus and pelvic diameters. The mechanism by which pelvic change occurs at birth is still not fully understood (Pavličev et al., 2019). And even the existence of such a change is debatable (Garagiola et al., 1989; Björklund et al., 1997).

In our study, we aimed to determine whether there is any change in SP width (SPW) during delivery and the factors associated with this condition if there is a change, as well.

Methods

This prospective observational study was performed at Gulhane Research and Education Hospital, Ankara, Turkey, between January 16, 2018, and June 25, 2018. The study protocol was approved by the Local Ethics Committee (Decision numbered 18/176). Written informed consent was obtained from all participants by the investigator (BC).

The women who had a singleton pregnancy at 37-42 weeks of gestation with cephalic presentation and were hospitalized for birth were included in the study. All women were Caucasian. Women with a prior cesarean delivery or any uterine operation, any SP separation history, or current symphysis joint pain were excluded.

Ultrasound examination was performed using a Voluson E10 ultrasound machine with a 3.5-MHz trans-abdominal transducer (GE Healthcare, Chicago, USA). SPW was assessed transabdominally (Figure 1), as described by Rustamova et al. (Rustamova et al., 2009) in each woman between the uterine contractions during the latent phase while cervical dilatation was 2 cm, the active phase while cervical dilatation was 6 cm and the second stage within one hour of complete cervical dilatation. The labor stages were determined according to Friedman's criteria. For the assessment of SPW, transabdominal transducer was vertically placed to the symphysial joint's upper border and then angled toward the lower abdomen, while the women were in supine position and her hips were flexed about 15°. So, a clear view of the upper part of the symphyseal joint was obtained in all women. The narrowest distance between the pubic bones where the medial pubic surfaces become parallel were measured as SPW. All the measurements were performed by senior author (KEK). To assess the reliability of the results, an experienced sonographer, who was blinded to study protocol and senior author's (KEK) results, repeated the measurements of the first 10 women. Test–retest series for the results in the first 10 women were done and showed good reliability.

Figure 1. Demonstration of symphysis pubis joint measurement

The demographic and obstetric data including woman's age, weight, length, gravida, parity, gestational age, ultrasonographic biparietal diameter (BPD) measurement of fetus in admission, birth weight of newborn, duration of active phase, the time between the full cervical dilatation and SPW measurement during second phase were recorded and entered into a statistical data base (SPSS, Version 17.0; Chicago, IL, USA) for analysis Kolmogorov-Smirnov test was used to analyze the normality of distribution of variables. Continuous variables with normal distribution are presented as mean ± standard deviation. Median (minimum-maximum) value is used where normal distribution is absent. Quantitative variables are given as number (percentage). One-way repeated measures analysis of variance (ANOVA)was used to analyze differences of the measurements obtained at the three intervals during labor. Tukey test was used to determine the differences between the individual groups in ANOVA. Pearson correlation coefficients were analyzed to evaluate the correlation between the measurements of SP and other continuous variables, including woman's age, weight, length, body mass index (BMI), gestational age, ultrasonographic BPD measurement in admission, birth weight of newborn, duration of active phase, the time between the full cervical

dilatation and SPW measurement during second phase. P <0.05 was accepted to be statistically significant.

Results

This study included 108 pregnant women who had been hospitalized for delivery. Table I shows the characteristics of them. The mean age and BMI were 29.71±5.45 years and 24.81±3.48 kg/m2, respectively. Sixty-six (61.11%) women were at nulliparous, while 23 (21.30%) were primiparous and 19 (17.59%) were multiparous. The mean ultrasonographic measurement of fetal BPD in admission and birth weight of newborn were 93.66±8.72 mm and 3580.19±358.23 g, respectively. The calculated length of active phase has a mean value of 524.07±254.99 minutes, while the mean time between the full cervical dilatation and SPW measurement in the 2nd stage of labor was 25.59±12.69 minutes.

In Table 2, the results of SPW measurement were shown. According to these, each value measured at 3 different times during delivery was statistically different from each other (All p values were <0.05). The mean SPW value measured in the latent phase (5.89±1.32 mm) was the smallest while the mean value in the second stage of the labor (7.59±1.55 mm) was the highest. In other words, the SPW value increased as the labor progressed.

In Table 3, the correlations between SPW measurements and other clinical factors were evaluated. As a result, it was found that only the birth weight was positively correlated each of SPW values measured at all three different times (for latent phase r=0.24, p=0.01; for active phase r=0.26, p=0.01; for second stage r=0.25, p=0.01; respectively). There was no correlation with other factors.

Table 1. Characteristics of the participants

Variables	**N= 108**
Woman's age (years)	29.71±5.45
Woman's length (cm)	165.18±5.76
Woman's weight (kg)	67.70±10.08
BMI (kg/m^2)	24.81±3.48
Gravida (number)	1 (1-5)
Parity (number)	0 (0-4)
multiparous	19 (17.59)
primiparous	23 (21.30)
nulliparous	66 (61.11)
Gestational age at delivery (days)	281.06±7.89
Biparietal diameter measurement (mm)	93.66±8.72
Birth weight of newborn (g)	3580.19±358.23
Duration of active phase (minutes)	524.07±254.99
SPW measurement time in the 2nd stage of labor (minutes)	25.59±12.69

Variables were given as mean ± standard deviation, median (minimum-maximum)
BMI: Body mass index, SPW: Symphysis pubis width

Table 2 Comparison of symphysis pubis width measurements

	Latent phase -I-	Active phase -II-	The second stage -III-	P	P_1 (I-II)	P_2 (I-III)	P_3 (II-IV)
SPW (mm)	5.89±1.32	6.53±1.42	7.59±1.55	<0.001	<0.001	<0.001	<0.001

Values were given as mean ± standard deviation
SPW: Symphysis pubis width
P<0.05 was statistically significant

Table 3. Correlations between clinical factors and SPW measurement

	Latent phase SPW		Active phase SPW		The second stage SPW	
	r	p	r	p	r	p
Woman's age	0.06	0.57	0.07	0.48	0.11	0.25
Woman's length	0.01	0.92	0.02	0.82	0.04	0.68
Woman's weight	0.12	0.21	0.09	0.34	0.08	0.40
BMI	0.12	0.20	0.09	0.36	0.07	0.47
Gestational age at delivery	0.17	0.08	0.19	0.06	0.17	0.07
BPD measurement	0.05	0.61	0.02	0.81	0.01	0.91
Birth weight of newborn	0.24	**0.01**	0.26	**0.01**	0.25	**0.01**
Duration of active phase	0.03	0.75	-0.11	0.25	-0.30	0.11
Measurement time in the 2nd stage	0.10	0.30	0.12	0.22	0.13	0.19

BMI: Body mass index, BPD: Biparietal diameter, SPW: Symphysis pubis width
r: Pearson's correlation coefficient
P<0.05 was statistically significant

Discussion

SP is a non-synovial joint connecting both pubic bones at midline in the anterior part of the pelvis and forms the anterior arch of the skeletal pelvis. It is interposed with a cartilaginous disc and supported by superior and inferior pubic ligaments. Its movements are extremely limited. However, SP can be more flexible and wider during pregnancy and labor, and after giving birth it returns to its former form (Alicioglu et al., 2008). In our study, the width of SP increased as labor progressed. In fact, this width was the least in the latent phase, and the highest in the second stage of labor. Our results are consistent with those of other studies who suggested that the SP may widen during labor (Björklund et al., 1997; Björklund et al., 1999; Rustamova et al., 2009). This may be an indicative of a physiological process. Labor is a process that occurs as a result of the appropriateness of certain characteristics of the mother and fetus. The fetus should be properly located in the birth canal and the fetus should be advanced to the outlet with sufficient maternal force. In addition, the pelvic structure must be suitable for delivery in order to achieve successful labor (Reitter et al., 2014). Increased SPW in the pelvis may also be an adaptation that widens pelvis and facilitates delivery.

Radiographic examinations have been used in similar very old studies and controversial results have been reported regarding the change of SPW during labor. It has been reported that this distance may increase or not change or even decrease (Thorp and Fray, 1938; Young, 1940). However, in more recent studies, it has been stated that ultrasonographic measurements can be used in the evaluation of SPW and this evaluation can be as reliable as radiographic evaluation (Becker et al., 2014). As a result of the use of ultrasonographic measurements, the idea that the width of SP increased during labor was accepted (Rustamova et al., 2009; Pavličev et al., 2019). However, it has been reported that these increase amounts are not very high (Rustamova et al., 2009). The results of our study supported this view and the amount of enlargement in SPW with the progression of labor differed by millimeters. During radiographic examination, correcting for magnification in images is difficult, because it is influenced by the distance of the measured object from the film. Therefore, examinations cannot be performed with subjects in a standardized position. Different postures may alter object-film distances, and change SP measurements. On the other hand, ultrasonographic examination ensures that the subjects' positions are standardized, is free of any risk and easy to use. Considering the low number of studies with ultrasonography in this topic, we think that our study may be useful for the literature.

When the relationship between SPW and various factors of labor was examined, we found that there was a positive correlation between SPW value and birth weight of the newborn for each period of labor. To the best of our knowledge, this finding is a new information for the literature Although this may support the presence of physiological changes in SPW to facilitate delivery as the weight of the newborn increases, it should be kept in mind that this may also be the result of increased mechanical load in the pelvis. We believe that further studies are needed to clarify this issue.

Our study has some limitations. The population in our study was relatively small and consisted of similar ethnic groups. We also did not evaluate the subjects' pelvic types in our study. Therefore, we were unable to determine whether genetical and

ethnical factors and pelvic structure may influence changes in the SPW during labor.

Conclusion

As a result, SPW increases as labor progresses. And this increase advances as the weight of newborn increases. These findings suggest that SP is a dynamic entity that facilitates labor. Nevertheless, further studies are needed to fully understand the effects of labor on SP and the effects of SP on labor.

Ethics Committee Approval:
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RESEARCH ARTICLE

Retrospective Evaluation of the Results of Elastic Intramedullary Nailing in Pediatric Femoral, Tibial, and Forearm Diaphyseal Fractures

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Abstract

Objective: In this study, we aimed to evaluate the clinical and radiological outcomes of pediatric femur, tibia, and diaphyseal fractures treated with elastic intramedullary nails.

Method: Overall 50 patients (34 male and 16 females; mean age: 7.7 years; range 5–14 years) diagnosed with femur, tibia, and forearm diaphyseal fractures were treated with titanium elastic nails (TEN). Of these, 18 were femur, 16 were tibia, and 16 were forearm diaphyseal fractures. One femoral shaft fracture was fragmented, and one tibia fracture was an open fracture. Open reduction was performed in two patients, whereas closed reduction was performed in the other patients. Anteroposterior (AP) and Lateral radiographies were used in the follow-up of patients. The patients were evaluated according to the range of motion, ability to use extremities, hospitalization duration, union duration of the fractures, inequality that may occur in the extremities, and development of complications. Flynn scoring system was used for evaluating the cases.

Results: Union of the fractures was achieved in an average of 6.9 (range: 4–11) weeks.A patient had a tibia fracture in the front-back plane AP with 5 varus angulation, 10 posterior angulation with a forearm fracture in the lateral plane, and 2.5 mm shortness was observed in an another forearm fracture. All patients, except one, had normal range of motion and could comfortably use the extremities. No nonunion, epiphysial growth arrest, and infection was observed in any patient. According to the Flynn criteria; perfect results were achieved in 34 patients and successful results were achieved in 16. Average union duration was 9.6 (range: 5–14) weeks, hospitalization duration was 3.5 (range: 3–6) days, and follow-up duration was 12.4 (range: 6–19) months.

Conclusions: Titanium intramedullary elastic nail is a treatment method that can be successfully performed on pediatric femur, tibia, and forearm diaphyseal fractures.

Key words: Titanium intramedullary elastic nail, pediatric femur diaphysis fracture, pediatric tibia diaphysis fracture, pediatric forearm diaphysis fracture, surgery

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Introduction

Pediatric fractures differ from adult fractures in terms of having more growth factor related environment, thicker periost, remodeling capacity, and less ligament injuries. These factors affect treatment approach. Many pediatric fractures are may be treated with conservative methods (Barry, 2004), However titanium elastic nail (TEN) are being used in the fixation of diaphysis long-bone fractures in adolescents and children (Lee, 2018).With great success .The biggest advantages of TEN are as follows: the minimally invasive nature of the technique, short operation time, and protection of the growth plate. Titanium and stainless steel are generally used in the production of elastic nails and both the metals have enough strength for adequate stabilization of the fractured bone (Mohamed, 2017).

In fractures that require operation, alternative treatment methods such as fixation with plate screw or external fixation may also be used successfully (Mohamed, 2017), however TEN allows slight movement in the fracture line and primary bone union without growth plate injury; it is a minimally invasive method that forms a small and esthetic scar tissue allowing early joint movement and normal physical activity, low infection rates, shortened psychological effects due to immobility, and shortened hospitalization duration,whichis the most important reasons that the method is widely accepted (Rajesh, 2016).

Intramedullary nailing in children with open epiphysis can be used in diaphyseal region fractures and some metaphyseal region fractures (Furlan, 2011) and additional immobilization with casting, usually not required postoperatively. In the present study, we aimed to evaluate the clinical and radiological outcomes of the titanium elastic nailing application performed on femur, tibia, and forearm diaphyseal fractures of children aged between 5–14 years.

Methods

The retrospective clinical study was approved by the Düzce University clinical research local ethics committee (decision numbered 236/2019). Overall 50 patients (34 male and 16 female; average age: 7.7,range 5–17 years)with 18 femur, 16 tibia, and 16 forearm diaphysis fracture diagnoses who were admitted to the emergency services at the Düzce University Faculty of Medicine Orthopedics and Traumatology clinic between May 1st, 2014 and October 31st, 2016, those aged > 5 and <14 years with femur, tibia, and forearm diaphysis fracture diagnosis who could not be stabilized with closed reduction and who were treated with TEN method were included. Sociodemographic and preoperative characteristics of the patients are shown in Table 1. All patients were treated with TEN.

Table 1. Sociodemographic and preoperative characteristics of patients

Preoperative Patient Data	Value
Number of patients	*50*
Male	34 (68%)
Female	16 (32%)
Average age (years)	.7 (5–14)
Etiology	
Simple falling	14 (28%)
Fall off of a bike	*15 (30%)*
Playing football	13 (26%)
Fall off the swing	4 (8%)
Traffic accident	4 (8%)
Fracture side	
Right	32 (64%)
Left	18 (36%)

Surgical Tecnique: Closed reduction under Scopy Control was attempted in all patients. After reduction, physis lines were determined. In femur fractures, the lower end of the femur was reached with two incisions in distal lateral, and medial metaphyseal regions. Two TENs were sent retrograde from holes opened from the lateral and medial region of the lower end of the femur to the subtrochanteric region passing through the fracture line (Figure 1a, b). In cases where reduction could not be achieved, fracture site was detected, and reduction was provided with possible minimal disection. In cases where adequate stability was not achieved, one more elastic nail was sent from the lateral side.

In tibia fractures; medial and lateral mini incisions were performed on 2 cm distal to tibial proximal epiphysis two elastic nails were sent through these holes till to the tibiaL lower end so as to pass the fracture line (Figure 2a, b).

In forearm fractures, the entrance point was determined from the lateral end of the distal physis using scopy for radius fractures. One TEN was sent in retrograde fashion from the opened hole. In case of an ulna fracture, proximal physis line was determined using again scopy and the nail was sent in antegrade fashion from the hole opened at the olecranon distal to physeal line (Figure 3a, b).

Figure 1a and b. Preoperative and Postoperative X-Ray Images of the Femur Fracture

Figure 2a and b. Preoperative and Postoperative X-ray Image of the Tibia Fracture

Figure 3a and b. Preoperative and postoperative X-ray images of the forearm fracture

Operated extremities of the patients were placed in braces for 2 weeks during the postoperative period. The patients were invited for routine follow-up at the postoperative 2nd, 4th, 8th, and 16th week and at 6th and 12th month. Ap-Lat radiographies were taken. Bony union decision was given when trabeculae or cortical bone crossing the fracture site was detected on radiographies and fracture site was stable and pain free during clinical examinations. The cases were evaluated in terms of hospital stay, fracture union duration, whether complications occurred, extremity length inequality, joint range of motion, and ability to use extremities. Clinical and radiological evaluation results were performed according to the titanium elastic nailing system of Flynn et al. (2001). This system was evaluated to have perfect, successful, and bad results according to length inequality (<1, 1–2, and>2 cm), alignment (<5°, 5°–10°, and>10°), pain (does not exist, does not exist, and exists), and complications (doesn't exist, minor-temporary, and major-permanent) (Table 2).

Statistical Evaluation

In our study, SPSS version 22.0 (SPSS, Chicago, Illinois, USA) was used for descriptive statistical methods (mean, standard deviation, frequency). These analyzes revealed that a sample of this size was sufficient to detect a clinically significant difference with 100%, 89%, and 95% power. Significance level was set at p = 0.05.

Table 2. Flynn criteria (2001)

	Perfect result	**Good result**	**Bad result**
Limb length discrepancy	<1.0 cm	1–2 cm	>2.0 cm
Angular deformity	<5°	5–10°	>10°
Pain	No	No	Yes
Complication	No	Minor-transitory	Major-permanent

Results

Of the fractures, 18 were femur, 16 were tibia, and 16 were forearm diaphyseal fractures. Union was achieved in all fractures in an average of 9.6 (range: 5–14) weeks. Average duration of hospital stay was 3.5 (range: 3–6) days. Average follow-up duration was 12.4 (range: 6–19) months.

One tibia fracture was an open fracture and one femur fracture was a comminuted fracture with butterfly fragment. Of the fractures, 14, 15, 13, 4, and 4 occurred due to simple falling, falling off of a bicycle, falling while playing football, falling off of a swing, and due to traffic accidents, respectively. There was head trauma in two cases. Open reduction was performed in two patients. A third elastic nail was sent from the lateral side in one femur fracture to provide adequate stability. Normal alignment was acquired in coronal and sagittal plane

in 48 patients, whereas 5° varus angulation was observed in a tibial bone fractured patient and 10° posterior angulation was observed in one of the forearm fractured patient; 2.5 mm shortness was observed in an additional forearm fractured patient. Slight limitation of hip and knee range of motion was in one of the femur fractured patient whıch became normal at his control 6 months post operatively. Nails were extracted from all patients in an average of 8 (range: 5–12) months. No delay in union, closing of growth plate (Epiphysis), and infection were observed. According to Flynn criteria, perfect outcome was achieved in 42 patients (84%) and successful outcome was achieved in eight (16%) ($p = 0.05$).

Discussion

In the recent years, the treatment of pediatric long-bone fractures with intramedullary nailing has been increasing. This is due to the technological advancements of the applied nails along with the changes made especially in elastic nails with the dominant attitude of the orthopedists involved in pediatric fractures (Barry, 2004). Primary advantages of elastic nails are as follows: early return to school after surgery, short hospital stays, low complication rates, remodeling, and fast bone healing without damaging the blood circulation of the physical plaque (Heinrich, 1992; Öztürkmen, 2002). Although it seems difficult to provide rotational stability and alignment during the elastic nail application, a closed reduction application accompanied by scopy control and careful aligment control during surgery minimizes complications (Aktekin, 2007). In the present study, open reduction was performed only in one tibial open fracture and one femural fragmented fracture. The most important disadvantages of these nails are as follows: inadequacy in providing rotational stability, shortness risk in fragmented fractures, bending of the nails from the fracture line, and discomfort at the nail entrance point (Kawalkar, 2018). Gogi et al. (2007) followed up pediatric patients with femur shaft fractures who were operated with elastic nails. They reported an average of 2.7-mm length increase in nine patients and an average of 11.7-mm decrease in four. They did not encounter any leg-length inequality in 10 patients. They reported that leg-length inequality is commonly observed, and it does not lead to functional problems over time. In the present study, no shortness was observed in any patient with femur fractures. Limitation was observed in hip and knee movements in one patient. The functions of this patient recovered during the follow-ups. No rotational alignment disorder clinically was observed in any patient. The treatment performed with TENs may cause nail-path infection or irritation. In the study by Onta et al. (2015) and in another study by Sankar et al. (2007), it was reported that 22% and 26% patients, respectively, have pin-path irritation. Kapil et al. (Kapil, 2016) reported that 13.3% patients had pin-path irritation and 4.4% patients had superficial pin-path infection. In our study, the nails embedded under the skin and caused irritation in three (6%) patients which were resolved by itself after the removal of TENs without causing any problems.

In pediatric forearm fractures, even the angulations >20° are corrected with remodeling children aged<10 years. However, in those aged >8–10 years, angulations >10° should not be accepted (Fuller, 1982; Larsen, 1988). Angulations >10° cause 3–4-mm relative shortness in the angulated bone, functional loss in the distal radio-ulnar joint, disruption of the interosseous distance, and limitation in the rotation movement of the forearm (Slongo, 2005; Kubiak, 2005). In the present study, a 10° posterior angulation was observed in one forearm fracture case and 2.5-mm shortness was observed in another forearm fracture case. However, no limitations were observed in the forearm rotation and wrist movements of the patients.

Generally, pediatric isolated tibia fractures are treated with conservative methods. Surgical indication is indicated by selected open fractures that cannot be reduced with closed reduction; those with multiple trauma and fractures; those with spasticity such as cerebral palsy, extreme soft tissue trauma, and compartment syndrome, and in children aged >10 years (Aktekin, 2007). Intramedullary nailing is not widely used in pediatric tibia fractures due to the triangular structure of the tibia, proximal and distal tibiofibular joints being located within the eccentric muscle structure, and its curve at the proximal end (Slongo, 2005). In all pediatric fractures, tibia fractures are the only ones that developed nonunion and malunion with elastic nail treatment according to slongo et al. (Slongo, 2005). In a previous study, elastic nail treatment achieved good results in the less fragmented open tibia fractures, which do not have segmental bone loss (Kubiak, 2005). In another study,50 pediatric tibia fractures were treated with intramedullary nailsanddelayed union was observed in 11% and nonunion in 14% patients (Gordon, 2007). Moreover, they detected that delayed union and

hypertrophic callous tissue formation increases with age. Leg-length inequality is the complication that may also occur after tibia diaphyseal fractures (Vallamshetla, 2006). They reported that 3.6% patients had leg-length inequality of <15 mm. In our present study, shortness did not develop in any tibia shaft fractures. In pediatric tibia fractures, 10° varus–valgus aligment in those aged <8 years and 5° varus–valgus in those aged >8 years are acceptable (Bilge, 2008). In the present study, 5° varus deformity developed in one patient but no problems were observed in their follow-ups.

A study evaluating 30 patients with tibia diaphyseal fractures reported that they achieved perfect, acceptable, and bad results at rates of 50%, 36%, and 14%, respectively (Debnath, 2017). In a study comparing patients that had either TEN or plate-screw osteosynthesis, it was reported that 97% had similar recovery (Pennock, 2017). In a study evaluating 16 patients (O'Brien, 2004), it was reported that all fractures completely healed without any pain within a 5-year period and no limitation of movement or refractures was observed. Furthermore, another study evaluating 19 patients with stable fractures, perfect, acceptable, and bad results were reported in 63%, 32%, and 5% patients, respectively (Sankar, 2007). Uludağ et al. achieved perfect and successful results in 60% and 40% of their patients, respectively (Uludağ, 2019). Another study conducted on 48 children using Flynn criteria (Govindasamy, 2018), the clinical results were evaluated, and perfect results were achieved in 42 children (83%) and adequate results were achieved in eight children (17%). In the present study, according to the Flynn criteria, perfect results were achieved in 42 patients (84%) and successful results in 8 (16%).

Using the elastic nail method, hospital stays, and related treatment costs significantly decreased according to Bilge et al. (Bilge, 2008). Average duration of hospital stays for our patients were 3.5 (range: 3–6) days and average follow-up duration was 12.4 (range: 6–9) months. In all fractures, union was achieved in an average of 9.6 (range: 5–14) weeks. In cases with plate application after pediatric long-bone fracture, plate needs to be removed after the fracture union. There is also a higher risk of re-fracture development after the removal of the plate This operation also causes relatively same amount of bleeding and trauma as the first operation. Moreover, due to the re-fracture risk after plate removal, patients are usually asked to use crutches for at least 3 weeks after removal. Thus, the activities of the patients during this period are limited due to the possibility of fracture. An important advantage of the TEN application is that the re-fracture risk is much lower than the plate application.

Intramedullary elastic nail application is an effective method used in pediatric long-bone fracture treatment. We believe that good results can be achieved with correct patient selection, appropriate surgical technique, and a good patient follow-up.

Conclusion

TEN can be successfully applied as a safe, effective, and low complication method in the treatment of pediatric femur, tibia, and forearm diaphyseal fractures aged between 5–12 years.

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RESEARCH ARTICLE

Bedside Ultrasonographic Evaluation of Skull Fractures in Pediatric Head Trauma Patients Admitted to Emergency Department

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Abstract
Objective: Among children with minor head trauma, the incidence of skull fractures is increased especially in those under one year of age. Several studies investigated the association between skull fracture and traumatic brain injury. In the present study, we aimed to test the potential of ultrasonography in detecting linear skull fracture in pediatric patients with minor head trauma.
Methods: Patients under the age of 18 years with minor head trauma who presented to the emergency units of Bozok University Faculty of Medicine or Ordu University Training and Research Hospital between March 1, 2019 and November 1, 2019 and were planned to undergo head CT by their responsible physicians were included in the present study. The exclusion criteria were a Glasgow Coma Scale (GCS) score <14, traumatic open skull deformity, depressed skull fracture, penetrating head trauma, neurological deficits, and hemodynamic instability.
Results: A total of 62 patients (%64,5 males) with a mean age of 7.29 years were included. The sensitivity and specificity of ultrasound in detection of fractures were %84.6 (% 95 CI: 65.13- 95.6) and % 94.4 (% 95 CI: 81.3-99.3), respectively.
Conclusion: In conclusion, we suggest that the use of ultrasound before CT examination in patients with minor head trauma may be a helpful means to detect cranial fractures.
Key words: Ultrasonographic, Pediatric Head Trauma Patients, Emergency Department

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Introduction

Pediatric head trauma remains to be an important public health problem as a common cause of mortality and morbidity in childhood (Verma et al., 2009; Yanagava and Sakamoto, 2009). Many children are admitted to emergency units with serious traumatic brain injury due to head trauma. The gold standard method to determine the need for surgical treatment is head computed tomography (CT). In addition, CT has an important role in demonstrating the extent of skull fractures and intracranial injury (Fundarò et al., 2012). Whereas the indications for CT are established in moderate

and severe traumatic brain injuries, there is no widely accepted assessment tool in the literature about the use of CT in minor head trauma (Siaarti, 2004; Klemetti et al., 2009; Sömez et al., 2018). In this context, physicians in emergency units may have difficulty deciding whether or not to perform head CT in patients with minor head trauma. Among the underlying causes of this difficulty are the inability of a routine physical examination in reflecting brain injury and the risk of radiation exposure, need for sedation, and increased cost associated with CT (Stein et al., 1991; Stein, 1992).

Among children with minor head trauma, the incidence of skull fractures is increased especially in those under one year of age (Greenes and Schutzman, 1997). Skull fractures can be classified as linear, depressed, and basilar and 75% of all skull fractures are linear in nature (Atabaki, 2007). Several studies investigated the association between skull fracture and traumatic brain injury (Greenes and Schutzman, 1991; Schutzman and Greenes, 2001; Boran et al., 2006). In addition, coexistence of a scalp hematoma with childhood head trauma is a possible indicator of skull fracture (Schutzman and Greenes, 2001; Greenes and Schutzman, 2001). From this point on, the detection of skull fractures in pediatric minor head trauma patients with scalp hematoma using ultrasonography may facilitate risk estimation and aid in the decision to perform further imaging (CT). In the present study, we aimed to test the potential of ultrasonography in detecting linear skull fracture in pediatric patients with minor head trauma.

Methods

This prospective study was started after Bozok University Ethics Committee approved the study with the approval code "2017-kaek-189_2019.02.28_24". Patients under the age of 18 years with minor head trauma who presented to the emergency units of Bozok University Faculty of Medicine or Ordu University Training and Research Hospital between March 1, 2019 and November 1, 2019 and were planned to undergo head CT by their responsible physicians were included in the present study. Other inclusion criterion was the presence of scalp hematoma, soft tissue swelling, or localized tenderness on the head because it is not feasible to screen the whole head for fractures by ultrasonography. The exclusion criteria were a Glasgow Coma Scale (GCS) score <14, traumatic open skull deformity, depressed skull fracture, penetrating head trauma, neurological deficits, and hemodynamic instability.

The HM70A with Plus ultrasound system (Samsung Medison Co., Ltd., Seoul, Korea) was used for ultrasonography and a 7-12 MHz linear probe was used to scan the skull for fractures. The ultrasonography examination was performed beginning on the areas with scalp hematomas, abrasions, and where focal tenderness was present. In case of scalp hematomas, the skull was examined in a perpendicularly oriented fashion on transverse and sagittal-coronal planes between the boundaries of the scalp hematoma. If the area to be examined is over a cranial suture, confirmation was performed from the contralateral area. Fracture lines, bone cortex irregularities, step-off sign, and free bone fragments were recorded. The location of fracture on ultrasound examination was compared with head CT images. Results of the ultrasound examination did not change the patients' diagnosis or treatment algorithm.

Statistical analysis

The categorical data were presented with numbers and percentages. We calculated the ultrasonographic performance characteristics including sensitivity, specificity, accuracy, positive and negative predictive values with 95% confidence intervals. The IBM SPSS 26 program (SPSS Inc., Chicago, IL) was used for statistical analyses.

Results

A total of 62 patients (64.5% males) with a mean age of 7.29 years were included. The distribution of patients with respect to age groups is shown in Table 1. The frontal bone was the most frequent site of trauma followed by the parietal, occipital, and temporal bones, respectively.

Table 1. Baseline characteristics

Study Group	n=62
Sex , n (%)	
Male	40 (64,5)
Female	22 (35,5)
Age , n (%)	
0<2	11 (17,7)
2<5	14 (22,6)
5<10	21 (33,9)
10<15	12 (19,4)
15<18	4 (6,5)
Injury site, n (%)	
Temporal	10 (16,1)
Occipital	11 (17,7)
Parietal	16 (25,8)
Frontal	25 (40,3)

On the CT examinations 26 of the patients (42%) were found to have fractures and the remaining 36 (58%) did not have fractures. Ultrasound examination achieved to detect fractures in 22 of these 26 patients and failed to show the fractures in the remaining four patients (Table 2) (figure 1). On the other hand, two out of 36 patients without a fracture on CT examination were reported to fractures on ultrasound examination. The sensitivity and specificity of ultrasound in detection of fractures were 84.6% (95% CI: 65.13- 95.6) and % 94.4% (95% CI: 81.3-99.3), respectively. The positive and negative predictive values and accuracy for ultrasound examination in detection of fractures were 91.7% (95% CI: 74-98), 89.4% (95% CI: 78-96), and 90.3% (95% CI 80.1-96.4), respectively.

Table 2. Comparison of ultrasound with tomography to determine skull fracture

	CT (+)	CT (-)
USG (+)	22	2
USG (-)	4	34
Sensitivity, % (%95 CI)	84.6 (65.1- 95.6)	
Specificity, % (%95 CI)	94.4 (81.3-99.3)	
Positive predictive value, % (%95 CI)	91.7 (73.9-97.7)	
Negative predictive value, % (%95 CI)	89.5 (77.5-95.5)	
Accuracy, % (%95 CI)	90.2 (80.1-96.4)	
p-Value*	<0.001	

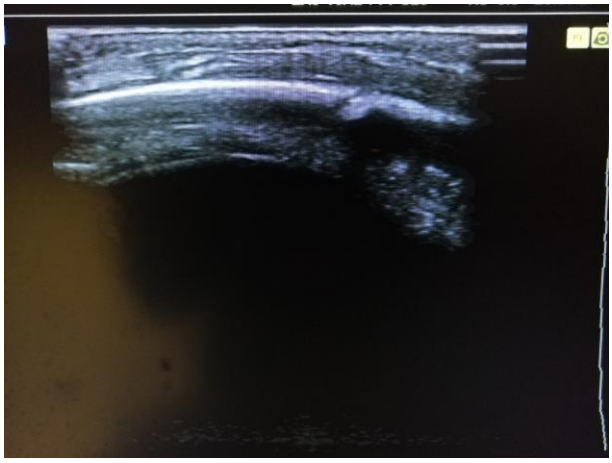

Figure 1. Ultrasonographic image of a six-year-old male patient with frontal skull fracture

Discussion

In the present study, we primarily demonstrated that ultrasonography is an effective tool to detect skull fractures in childhood minor head trauma. Secondly, we found that the specificity of ultrasound was higher than its sensitivity in detecting skull fractures, hence ultrasound seems to be more effective in excluding fractures in patients with minor head trauma. However, the low prevalence of fractures in the present study may also be the cause of this high specificity. It has been previously reported that specificity increases in case of a low prevalence (Brenner and Gefeller, 1997). Therefore, the prevalence rates should be taken into consideration when using ultrasound to detect/exclude cranial fractures.

In the literature, the number of studies regarding the detection of skull fractures using ultrasound are low. In one study, 69 patients under the age of 21 years were included and eight of these patients (12%) were found to have fractures (Rabiner et al., 2013). The sensitivity and specificity of ultrasound in detecting fractures were 88% (95% CI 53-98) and 97% (9%5 CI: 89-99), respectively. In another study, 55 patients under the age of 18 years underwent bedside ultrasound examination and 35 of these patients (63.6%) were found to have fractures (Parri et al., 2013). The sensitivity and specificity of ultrasound in detecting fractures were 100% (95% CI 88.2-100) and 95% (9%5 CI: 75-99.9), respectively. Another study in 46 subjects under the age of 18 years, 11 of the study population (24%) were found to have fractures (Riera and Chen, 2012). In that study, the sensitivity and specificity of ultrasound in detecting cranial fractures were 82% (95% CI: 48- 98) and 94% (95% CI: 79-99), respectively. In the present study, the sensitivity and specificity of ultrasound in terms of cranial fracture detection was comparable with the findings of the aforementioned studies. However, ultrasound is an operator dependent modality and there is need for large scale studies to reach a conclusion regarding the exact role of ultrasound in cranial fracture detection.

Ultrasound has many advantages over CT such as the absence of radiation risk, repeatability, and cost-effectiveness (Weinberg, 2010). Sedation of the patient may be required for CT imaging in the pediatric population. Sedation of young children may increase the risk of complications and may cause difficulty in surveillance by affecting the state of consciousness (Sanborn, 2005). On the other hand, ultrasound examination does not require sedation and may facilitate clinical follow up of

these children by emergency unit physicians. Another advantage of using ultrasound is that it may be performed at bedside and obviates the need to move the patient from the emergency unit (Coskun, 2018). We think that these advantages may prove helpful in early and accurate decision making by emergency unit physicians.

The primary task of the emergency physician in head trauma is to determine the presence of life-threatening problems. Current clinical and physical examination findings of the patients may be inadequate in the management of patients with minor head trauma. In the recent years, the rates of unnecessary head CT examination in head trauma patients is rather high and most of these examinations do not reveal pathological findings that necessitate surgical intervention (Blackwell et al., 2007; Atmis et al., 2016; Zulfiqar et al.,2017). In a study, only 6% of patients with minor head trauma were found to have pathological findings on CT (Mannix et al., 2012). In another study, the authors stated that approximately 69% of patients with minor head trauma did not require CT examination (Fundaro et al., 2012). In another study, 90% of head CT examinations were reported as normal in patients with minor head trauma (Atabaki, 2007). Performance of CT scans in children with minor head trauma should be reduced to protect children from the hazardous effects of radiation. However, widely accepted management algorithms are not available at the present (Er et al., 2013). We suggest that performance of ultrasound in minor head trauma patients may obviate the need for a CT examination when no fracture is detected on ultrasound. In addition, the detection of a fracture on ultrasound examination may prompt the physician to perform urgent further imaging.

On some occasions a CT device may not be available in the hospital and conventional radiography may be preferred in these centers. However, several studies indicate that the specificity and sensitivity of conventional radiography is low in minor head trauma patients (Feuerman et al., 1988; Thiruppathy and Muthukumar, 2004). The management of a patient with minor head trauma in a center without a CT device is controversial and it is hard to determine which patient to discharge home because of low risk and which patient to refer for further examination and treatment. However, although ultrasound examination may aid in the management of patients with head trauma by detecting or excluding fracture, its exact role is not clear. We found a relatively high sensitivity and specificity for ultrasound in patients with minor head trauma, but further larger scale studies are needed to reach a strict conclusion in this topic.

The most important limitation for ultrasound to detect cranial fractures in patients with scalp hematoma is the fact that the fracture line may not be under the scalp hematoma and rather near the boundary of it (Arnholz et al., 1998). Another limitation is when a tiny fracture connects with a cranial suture (Furuya et al., 1984). In the present study, the fracture line was near the boundary of scalp hematoma on CT in a patient in whom ultrasound failed to detect the fracture. Another two patients in whom ultrasound failed to detect the fracture had linear nondisplaced thin fractures. A sound knowledge of cranial suture anatomy may avoid this type of confusion. As a matter of fact, in the present study, the ultrasound operator evaluated the suture line of these two patients as fractures. Patient cooperation is also important for an effective screening. The ultrasound operator should carefully scan the area in all directions. In the present study, one patient in whom the fracture could not be detected by ultrasound examination had a poor cooperation. Another difficulty in ultrasound scanning is the application of ultrasound gel on the hair. Fluid-filled gloves or special pads may be used to overcome this problem. However, when mirror-image artefact should be kept in min when using a fluid-filled device on a scalp hematoma.

Conclusions

In conclusion, we suggest that the use of ultrasound before CT examination in patients with minor head trauma may be a helpful means to detect cranial fractures. We also believe that many unnecessary CT examinations may be avoided in patients with minor head trauma if an appropriate scoring system is established. We also believe that ultrasound examination would play a key role in the risk calculation and management in patients with minor head trauma provided that its exact role in this condition is established with further studies. We believe that our work would shed light on future studies in this topic.

Ethics Committee Approval: Ethics committee approval was received for this study from Yozgat Clinical Research Ethics Committee of Bozok University (Ethichs No: 2019-01-07).
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Zulfiqar M, Kim S, Lai JP, Zhou Y. The role of computed tomography in following up pediatric skull fractures. American journal of surgery. 2017;214(3):483-8..

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RESEARCH ARTICLE

Investigation of the Effects of Sun-Dried Apricot with Different Sulphur Levels on Oxidative Stress Markers

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Abstract

Objective: Apricots are mostly consumed in dried form worldwide. Studies are reporting some allergic effects of dried apricot because of its content of sulfur, which is used in the drying process. Therefore, sulphur content has been limited by many countries. This study was conducted to determine the effects of sun-dried apricots with different sulfur contents on nitric oxide (NO), malondialdehyde (MDA) and glutathione (GSH) that are among the markers of oxidative stress.

Methods: A total of 84 Wistar albino rats were divided into groups as control, sun-dried apricot, 1000, 2000, 3000 and 4000 ppm sulfured groups. At the end of the 12th week, rats were sacrificed under general anesthesia. NO, MDA and GSH levels were studied at the end of the experiment from the ovarian and testicular tissues of the rats.

Results: No statistically significant differences were found among the groups in terms of NO, MDA and GSH in the ovarian and testicular tissues removed from the rats

Conclusion: We claim that sulfur amount of dried apricots is not affect on reproductive system. This study is guiding further studies that will be conducted against the restrictions in sulfur used in the drying process of dried apricots, which is economically very important for dried fruits sector.

Key words: Sulphur dried apricot, Oxidative stress, Rat, Ovary, Testes

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Introduction

Today studies conducted on herbals that can potentially replace medical drugs are important because of the wide range of side effects. Apricot is one of the fruits investigated for alternative or complementary treatments of various diseases. Apricot, which belongs to the Rosaceae family is a medical plant with reported contents polyphenol, polysaccharides, fatty acid and carcinogenic glycosylase, vitamin A and vitamin C. Apricot also contains basic flavonoids such as chlorogenic acids, catechins and quercetin (Chan et al., 2008). Parlakpinar et al. found that feeding with apricots reduces ischemia-reperfusion damage in rats (Parlakpinar et al., 2009). Antimicrobial, cardioprotective, antimutagenic and anti-inflammatory activities and hepatoprotective effects

of apricot have been reported (Raj et al., 2016; Ruiz et al., 2005; Yılmaz et al., 2015).

Apricot is most commonly used in dried form. Philippi reported that nutrients, and thus calorie concentration are higher in dried apricot (Zamboni et al., 2011). Dried apricot is one of the important traditional export products of the dried fruit sector, and Turkey is the leading country of fresh and dried apricots production worldwide. In Malatya province, which meets more than half of the apricot production of Turkey, the production is more commonly wide with dried apricots and 90% to 95% of dried apricots are exported (TCEB, 2017).

The drying process aims to prolong shelf life by reducing physical, chemical, microbiological and enzymatic reaction rates through the removal of the water content, to decrease costs of packaging, warehouse and transportation, and to protect nutritional value (Jayaraman and Das Gupta, 1992). Another drying method, drying by sulphurization is preferred to protect the natural color of apricots and to prolong shelf life (Bayram et al., 2007).

It has been proven that sulfur dioxide (SO_2) which is used in the drying process may lead to oxidative stress and DNA damage in various organs (Meng and Liu, 2007). Also, it has been reported that SO_2 /sulfide may cause biological damage by commencing peroxidation of polyunsaturated fatty acids (PUFA), DNA synthesis accompanied by mutations, cellular division and inhibition of mitosis (Shapiro, 1977). Again, it has been underlined that sulfide-based preservatives, especially in apricots and some dried fruits, may cause allergic reactions or asthma exacerbations in susceptible persons.

Based on these studies, it has been proposed that the content of the sulfur used in drying foods should be reduced. The legal limit of SO_2 content in dried apricot has been accepted as 2000 mg/ kg in European Union, 2500 mg/kg in Canada and 3000 mg/ kg in the United States (Asma, 2007; Codex, 1981).

Estimated amounts of daily intake of inorganic food additives per person are reported 1.629 mg (as 50 kg body weight) for sulfur dioxide (Ishiwata et al., 1998). It is important to establish the balance between sulfur level used in the dried fruits to protect natural color and prolong shelf life, and biochemical reactions caused by this agent. The number of studies in the literature on this issue is not sufficient. Because oxidative stress that has been proposed to be increased by sulfur, which is used in the drying process of apricots plays an important role in the pathophysiology of many diseases, we thought that investigation of the effects of sulfur levels used in the drying process of apricot on the markers of oxidative stress will clarify this issue. Therefore, based on this information, in this study, we aimed to investigate the effects of sun-dried apricot with various sulfur levels on NO, MDA and GSH that are among the markers of oxidative stress.

Methods

Chemicals, animals and diets

A total of 84 Wistar albino rats used in this study (42 female and 42 male) were supplied from the Inonu University Experimental Animals Reproduction and Research Center. The principles of the Inonu University Experimental Animals Ethics Committee was followed during the study. 18-weeks old rats weighing 205 ± 13 g were kept in standard cages until the day of the experiment. Throughout the experiment, the drinking water was changed daily, and the standard cage cleaning was done. The rats were housed in rooms with air conditioner in 24-27 °C room temperature with 12 hours of light and 12 hours of dark.

Female and male rats were kept in separate cages and divided into 6 groups. Each group included 7 rats to provide statistical significance. The rats were distributed into the cage to provide a maximum of two rats in each cage to provide homogenous feed intake. Only control groups were fed with commercial pellet feed.

The rats used in this study were divided into groups as follows.

Group 1: Control Group (n=14; 7 males, 7 female): Rats were fed with commercial pellet feed and sacrificed in the 12th-week.

Group 2: Sun-Dried Apricot Group (n=14; 7 males, 7 female): Rats were fed with feed prepared with (w/w) 10% dried apricot ad libitum and sacrificed in the 12th-week

Group 3: 1000 ppm Sulfured Apricot Group (n=14; 7 males, 7 female): Rats were fed with feed prepared with (w/w) 10% dried apricots containing 1000 ppm sulfur ad libitum and sacrificed in the 12th-week.

Group 4: 2000 ppm Sulfured Apricot Group (n=14; 7 males, 7 female): Rats were fed with feed prepared with (w/w) 10% dried apricots containing 2000 ppm sulfur ad libitum and sacrificed in the 12th-week.

Group 5: 3000 ppm Sulfured Apricot Group (n=14; 7 males, 7 female): Rats were fed with feed prepared with (w/w) 10 % dried apricots containing 3000 ppm sulfur ad libitum and sacrificed in the 12th-week.

Group 6: 4000 ppm Sulfured Apricot Group (n=14; 7 males, 7 female): Rats were fed with feed prepared with (w/w) 10 % dried apricots containing 4000 ppm sulfur ad libitum and sacrificed in the 12th-week.

Determination of Sulfur Amount of Apricots

The sulfur amount in the apricots was determined according to the Turkish Standard no:8131. The apricots were classified according to the sulfur content, ground and stored at +4^{o}C in portions.

Preparation of Pellets Containing Sun-Dried Apricots and Sulfur-Containing Apricots

During the experiments, sun-dried and sulfur-containing apricots were mixed with pellets at 10%. In order to minimize the decrease of sulfur ratio due to waiting for the prepared pellets, the pellets were prepared as 3 kg at every turn. For this purpose, 300 g of the apricots that were previously ground and kept at +4 ° C were weighed and powder pellet was added until 3 kg pellet was obtained (approximately 2700 g). Some water was added to the mixture, which was then worked until it became thoroughly homogenized and then made pellet again utilizing a pellet machine.

Obtaining Ovarian and Testicular Tissues and Preparing for Analyzes

At the end of the 12th-week, rats were sacrificed under general anesthesia. Ovarian and testicular tissues from rats were wrapped in aluminum foil and stored at -70 °C in deep freezing until the day when biochemical tests were to be carried out.

Tissue Homogenization and Tampons

Tissues weighed approximately 200 mg were homogenized at a rate of 16000 rpm by adding 2 mL of Tris - HCl tampon (pH: 7.0). After homogenization, the tubes were centrifuged at 4000 rpm for 10 minutes at +4 °C. Supernatants formed after centrifugation were taken into Eppendorf tubes and kept in the freezer until the day of operation. All chemicals and reagents used were of analytical grade and were purchased from Sigma-Aldrich (Saint-Quentin-Fallavier, France). Bidistilled water was used in all the studies.

Estimation of Oxidative Stress Markers

Measurement of Nitric Oxide

NO formed in the medium by nitric oxide synthase (NOS) activity was reduced from nitrate to nitrite with cadmium granules, and the colored compound formed following reaction with Griess reactive was measured at 545 nm with a spectrophotometer (Cortas and Wakid, 1990).

Measurement of Lipid Peroxidation

The measurement of MDA, which is a product of lipid peroxidation was performed using the Uchiyama and Mihara method (Mihara and Uchiyama, 1978). This method is based on spectrophotometric evaluation of the absorbance of pink-red color formed as a reaction of tissue MDA with 2-Thiobarbituric acid (TBA) at 95^{o}C. For the standard measurement, the standard graphic was drawn with solutions prepared by serial dilution of the 10 mmol/L $NaNO_3$ stock solution (5-200 µM).

Measurement of Reduced Glutathione

This method is based on spectrophotometric evaluation of the absorbance of yellow product formed as a reaction of sulfhydryl groups found in glutathione with Ellman's reagent 5,5′-Dithiobis (2-nitrobenzoic acid) (DTNB), at 412 nm (Tietze F, 1994).

Statistical analysis

In the statistical analysis of the data obtained from this study, normality of the variables was tested with the Shapiro-Wilks method and it was found that the variables were non-normally distributed. Thus, the variables were evaluated using non-parametric tests. Kruskal Wallis test was used in the comparison of groups and Mann-Whitney U test for paired comparisons. $p<0.05$ values were considered statistically significant. The statistical analysis was performed using IBM SPSS Statistics version 23.0 package software.

Results

A Total of 84 Wistar albino rats were included in the study with 42 being female and 42 males. The mean age of the rats was 18 weeks and the mean weight was 205 ± 13 g. Ovarian and testicular tissues obtained from the sacrificed rats at the end of the 12-week experimental period were subjected to biochemical analysis with the spectrophotometric method.

When oxidative stress markers were examined in the ovarian tissues obtained from the female rats

were examined; median NO values were found as 38.95 µmol/g in Group 1, 41.51 µmol/g in Group 2, 44.25 µmol/g in Group 3, 37.89 µmol/g in Group 4, 44.70 µmol/g in Group 5 and 43.33 µmol/g in Group 6. In the Kruskal Wallis test, no significant difference was found among the groups in terms of NO values (p=0.733) (Figure 1).

Median MDA values were found as 43.43 nmol/g in Group 1, 41.14 nmol/g in Group 2, 43.18 nmol/g in Group 3, 42.02 nmol/g in Group 4, 44.42 nmol/g in Group 5 and 43.37 nmol/g in Group 6. No statistically significant difference was found among the groups in terms of MDA values (p=0.975) (Figure 1).

Median GSH values were found as 39.82 µmol/g in Group 1, 38.34 µmol/g in Group 2, 36.19 µmol/g in Group 3, 32.85 µmol/g in Group 4, 31.98 µmol/g in Group 5 and 31.19 µmol/g in Group 6. No statistically significant difference was found among the groups in terms of GSH values (p=0.659) (Figure 1).

When oxidative stress markers were examined in the testicular tissues obtained from the male rats were examined; median NO values were found as 35.99 µmol/g, 40.87 µmol/g in Group 2, 43.10 µmol/g in Group 3, 41.72 µmol/g in Group 4, 44.70 µmol/g in Group 5 and 42.42 µmol/g in Group 6. In the Kruskal Wallis test, no significant difference was found among the groups in terms of NO values (p=0.365) (Figure 2).

Median MDA values were found as 45.65 nmol/g in Group 1, 43.34 nmol/g in Group 2, 45.93 nmol/g in Group 3, 43.78 nmol/g in Group 4, 45.10 nmol/g in Group 5 and 44.08 nmol/g in Group 6. No statistically significant difference was found among the groups in terms of MDA values (p=0.679) (Figure 2).

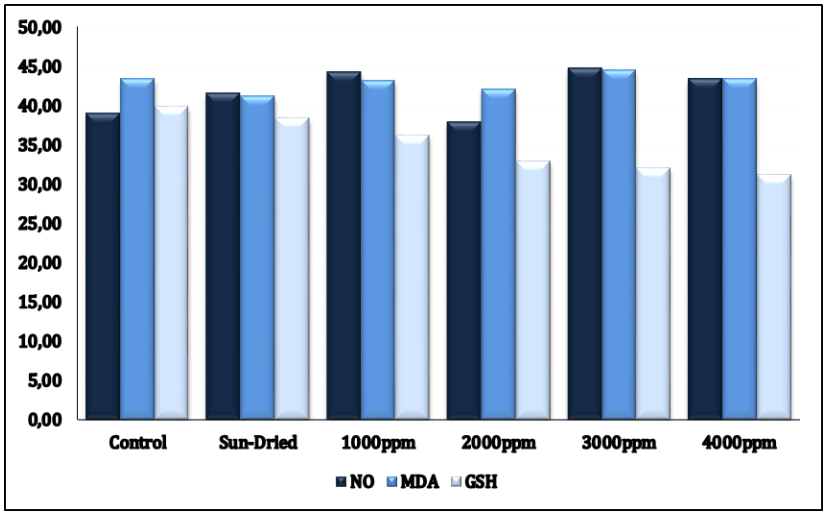

Figure 1. NO, MDA and GSH values in ovarian tissues obtained from the groups.

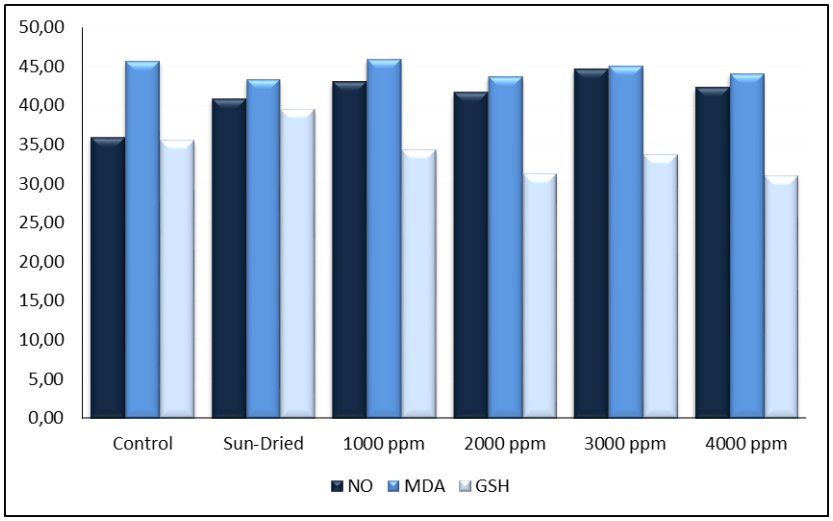

Figure 2. NO, MDA and GSH values in testicular tissues obtained from the groups

Table 1. Comparison of oxidative stress markers.

Tissue	Groups	N0 (µmol/gr wet Tissue) Median (min.-max.)	MDA (nmol/gr wet tissue) Median (min.-max.)	GSH (µmol/gr wet tissue) Median (min.-max.)
OVER	Control	38.95 (31.08-46.83	43.43 (32.01-63.07)	39.82 (21.56-45.47)
	Sun-dried	41.51 (36.19-51.62)	41.14 (38.72-48.95)	38.34 (35.05-45.07)
	1000ppm	44.25 (34.67-52.06)	43.18 (34.24-62.05)	36.19 (28.33-42.17)
	2000ppm	37.89 (33.93-65.28)	42.02 (34.28-63.43)	32.85 (27.81-46.08)
	3000ppm	44.70 (37.86-56.05)	44.42 (38.72-61.60)	31.98 (30.09-49.38)
	4000ppm	43.33 (34.41-63.86)	43.37 (34.47-65.18)	31.19 (27.62-48.81)
	p	***0,733***	***0,975***	***0,659***
TESTES	Control	35.99 (31.22-45.23)	45.65 (41.36-54.63)	35.64 (22.28-41.80)
	Sun-dried	40.87 (33.43-48.43)	43.34 (36.49-53.46)	39.53 (32.85-43.12)
	1000ppm	43.10 (36.72-50.51)	45.93 (38.98-52.80)	34.38 (30.01-44.70)
	2000ppm	41.72 (32.21-58.75)	43.78 (38.24-62.04)	31.35 (28.31-42.35)
	3000ppm	44.70 (37.04-49.93)	45.10 (42.02-52.50)	33.73 (27.89-42.90)
	4000ppm	42.42 (33.81-55.56)	44.08 (38.44-56.43)	31.04 (21.48-49.17)
	p	***0,365***	***0,679***	***0,572***

Median GSH values were found as 35.64 µmol/g in Group 1, 39.53 µmol/g in Group 2, 34.38 µmol/g in Group 3, 31.35 µmol/g in Group 4, 33.73 µmol/g in Group 5 and 31.04 µmol/g in Group 6. No statistically significant difference was found among the groups in terms of GSH values (p=0.572) (Figure 2). A comparison of oxidative stress parameters within groups was given in Table1.

Discussion

Apricot consumption is predominantly in dried form worldwide. Sulphurization process is performed in drying apricots to provide fruit supply and prolong shelf life. According to the results of the study by Davis et al., dried fruits suppliers should target 3000 ppm SO_2 content for an acceptable fruit supply from one season to another (Davis et al., 1973).

On the other hand, the sulphurization process has been reported to affect many body mechanisms, especially oxidative stress. Lizada et al. demonstrated that sulfur initiates linoleic acid peroxidation through a free radical mechanism (Lizada and Yang, 1981). It has been shown that sulfides lead some health problems such as asthmatic reactions when inhaled or intaken to the body in susceptible persons (Miranda et al., 2009). Besides, sulfur causes allergic reactions in susceptible people. These allergic reactions are in a wide range from mild reactions such as urticaria to severe reactions such as difficulty in breathing and potential anaphylactic shock. Also, low blood pressure, abdominal pain and diarrhea can be seen in susceptible persons.

Therefore, the sulfur content in dried fruits, including apricots has been restricted in many regions of the world. Sulfur content in dried fruits has been limited between 1000-3000 ppm in many countries. While the Australia government has a limited amount of sulfur in foods with 3000 ppm, England has determined this limit as 2000 ppm. In Turkey, which is among the largest apricots suppliers worldwide, one of the most important problems for the dried fruit sector is a limited amount of sulfur in terms of export.

According to the CODEX General Standard for Food Additives, the maximum sulfur level allowed for dried apricot is 2000 ppm. This rate is also 2000 ppm in the Europe Union directive 95/2/EC (Codex). The amount of sulfur and duration of sulphurization are the effective parameters on sulfur content due to these limitations (Turkyılmaz et al. 2013). Research on alternative drying methods is continuing.

On the other hand, studies are reporting that a large part of sulfur absorbed during sulphurization losses during drying and again there is a slow loss in sulfur content during shelf life (McBean, 1967).

There is no study in the literature investigating the effects of sun-dried apricots with various sulfur levels on body mechanisms. There are only few articles that investigate the effects of sulfur exposure in workers. Koksal et al. found significant decrements in FEV1, FEV1/FVC%, and FEF 25 –

75% in study of pulmonary function in 69 workers revealing that SO_2 exposure on the airways of the workers gas may be the causative agent of asthma-like syndrome (Koksal et al., 2003). In a vivo study by Koksal et al., found that cytokines such as TNF-a, IL-1h, IL-6, IL-8 and NO may play a role in the pathogenesis of SO_2-induced bronchoconstriction in the asthma-like syndrome caused by high concentration of SO_2 exposure during apricot sulfurization processes (Koksal et al., 2003a). Unlikely these results, in an in vivo study Knorst et al. found that high amount of sulfur dioxide induced a significant decrease in spontaneous and lipopolysaccharide-stimulated tumor necrosis factor-α and lipopolysaccharide-stimulated interleukin-1β release (Knorst et al., 1996) But in our study, no statistically significant differences were found between NO, MDA and GSH levels that are among oxidative stress markers in the ovarian and testicular rats that were fed with non-sulfured and 1000, 2000, 3000 and 4000 ppm sulfured sun-dried apricots over 12 weeks (Table1). Given significant economical losses about dried apricots supply and export, our results indicate an important lack of studies to be conducted on this issue. We could not compare our results with other studies since there is no study on the same issue.

As a result, we haven't seen any significant changes, even in 4000 ppm group, in oxidative stress parameters of rats' ovarian and testicular tissues. We claim that sulfur amount of dried apricots is not affect on reproductive system. This study is guiding in the literature against the restrictions in the drying process of dried apricots, which is economically very important for the dried fruits sector. We believe that the results of our study are encouraging for furthermore comprehensive studies that will be performed in the future.

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Author Contributions: Concept – TRK, OO, EK Design TRK, EK Supervision TRK, EK, OO; Materials –TRK, EK, OO; Data Collection and/or Processing – TRK, OO, EK, NO; Analysis and/or Interpretation- TRK, OO, EK, NO; Literature Review- TRK, OO, EK, NO; Writing- TRK, OO, EK, NO; Critical Review - TRK, OO, EK, NO.

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RESEARCH ARTICLE

Demodex spp Distribution in Patients with Alcohol Abuse

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Abstract

Objective: Demodex is an ectoparasite that can infest the human body. The infestation can progress rapidly when the immune system is suppressed. The condition has also been reported to be more common in subjects with alcohol abuse because of the associated immune system abnormalities and decreased hygiene. Our aim in this study was to determine whether a relationship was present between Demodex spp. infestation and alcohol abuse

Methods: A total of 26 patients diagnosed with alcohol abuse according to DSM-5 at the psychiatry outpatient department and 24 age- and gender-matched healthy volunteers who met the study inclusion criteria were included in the study. A standard superficial skin biopsy (SSSB) was used in the diagnosis of Demodex folliculorum. The compliance of the numeric variables with the normal distribution was investigated with the Shapiro-Wilk test. The data were presented as mean, standard deviation and percentages

Results. A significant relationship was found between Demodex presence and alcohol abuse (p=0.001). The relationship was also significant when patients with both alcohol abuse and smoking were investigated (p<0.001).

Conclusion: The Demodex spp. infestation incidence may be increased in patients with alcohol abuse. We recommend providing the relevant treatment especially for erythematous and itchy facial lesions in these patients as Demodex spp. can be opportunistic pathogens.

Key words: Demodex, Alcohol abuse, smoking, addiction and demodex.

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Introduction

Demodex is from the Demodicidae family of the Trombidiformes genus and 8 species and 16 types that are parasitic in mammals are known. Only two types of Demodex can infest the human body. *Demodex folliculorum* (*D. folliculorum*) can be present in hair follicles by itself or in groups. *Demodex brevis* (*D. brevis*) is found in sebaceous glands and is usually detected by itself. The parasite can be found on the face, scalp, neck, ears, chest, nipples and genital region, in order of incidence. It has been reported to spread by close contact, shaking hands and kissing (Karaman et al. 2016; Turan et al., 2017).

The density of Demodex mites in cm2 in the normal skin is less than 5. They can cause skin disorders when present in numbers over 5 in a pilosebaceous unit (Yun et al., 2017). The parasite incidence has been reported to be influenced by the skin pH, moisture, temperature and personal hygiene, all of which affect the density (Tilki et al., 2017). The parasite may also play an important role in the pathogenesis of various disorders such as rosacea, acne vulgaris, dermatitis and blepharitis (Yun et al. 2017).). Routine diagnostic methods such as cellophane slides, skin scraping, punch biopsy of lash samples and standard superficial skin biopsy are used in the diagnosis (Aycan et al., 2006; Kockaya, 2016; Yun et al. 2017).

Demodex infestation can show severe progression in immunosuppressed patients (Ozcelik, 2007). Chronic alcohol use has also been reported to have a negative effect on immune system functions (Crews, 2006; Romeo, 2007). Demodex infestation can be more common in subjects with alcohol abuse as immune system abnormalities and decreased hygiene can be present (Kockaya, 2016). A literature survey has revealed that the epidemiology of Demodex species has been studied in subjects with alcohol abuse. We therefore tried to determine whether a relationship was present between *Demodex* spp. infestation and subjects diagnosed with alcohol abuse in the psychiatry outpatient department.

Methods

Ethics Committee permission was obtained before the study started. A consent form was obtained from the patients who were attending the psychiatry outpatient department for alcohol abuse and who accepted to provide samples. A total of 26 subjects with alcohol abuse and 24 healthy volunteers were included in the study. The healthy volunteers were age- and gender-matched with the patients and did not smoke or use alcohol or any other substance. Those with known systemic or dermatologic diseases were not included in the study

A standard superficial skin biopsy (SSSB) was used in the diagnosis of D. folliculorum. The method is non-invasive and is performed by taking the surface layer of the skin and the follicle content with the help of cyanoacrylate adhesive (Marks and Dawber, 1971). A Demodex density over 5/cm2 with SSSB is reported to be significant for the final diagnosis (Marks and Dawber, 1971; Erbagci and Ozgoztasi, 1998). In practice, a drop of cyanoacrylate adhesive is placed on a clean slide. Mild pressure is applied with the slide to the relevant facial region and the adhesive contacts the face for one minute. The slide is then withdrawn with a single movement and 2 to 3 drops of Hoyer solution is placed on the sample. The sample is then covered with a coverslip and examined under the light microscope at 4x and 10x magnification. Five or parasites in a one cm2 area was accepted as proof of infestation (demodicidosis) in this study.

Statistical evaluation

The IBM SPSS Statistics 25.0 Program was used. The compliance of the numeric variables with the normal distribution was investigated with the Shapiro-Wilk test. The data were presented as mean, standard deviation and percentages. The Chi-square test was used for categorical data. The independent two-sample t test was used for the comparison of groups for numeric variables.

Results

The rate of parasite detection in the subjects with alcohol abuse and the normal volunteers is presented in table 1.

Table 1. *Demodex spp.* Distribution Rates

			Patient	**Control**	**p***
Demodex		Present	13 (50%)	2 (8.3%)	0.002
		None	13 (50%)	22 (91.7%)	
		Total	26 (100%)	24 (100%)	
Gender		Female	2 (15.38%)	2 (16.66%)	0,933
		Male	24 (84.62%)	22 (83.34%)	
	Group	**n**	**Mean**	**Standard Deviation**	**p***
Age	**Patient**	26	44.69	9.785	0.626
	Control	24	43.33	9.814	
	N	**Minimum**	**Maximum**	**Mean**	**Std. Deviation**
Time(Year)	26	1	40	18.12	11.476

p*: Chi-square test

Table 2. Demographic Data of the Patient and Control Groups

		N	Yüzde (%)
Gender	Male	46	92.0
	Female	4	8.0
	Total	50	100.0
Demodex	Yes	15	30
	No	35	70
	Total	50	100.0
Smoking	Yes	23	46
	No	27	54
	Total	50	100
Alcohol Abuse	Yes	26	52
	No	24	48
	Total	50	100
Skin Findings (Patient Group)	Yes	17	34
	No	9	18
	Total	26	52

Table 3. The distribution of Demodex according to alcohol abuse, smoking status and skin findings

			Alcohol Abuse		Smoking		Skin findings	
			Yes	No	Yes	No	Yes	No
Demodex	No	Number	13	22	25	10	7	6
		%	37.1	62.9	71.4	28.6	53.8	46.2
		Total %	26.0	44.0	50.0	20.0	26.9	23.1
	Yes	Number	13	2	2	13	2	11
		%	86.7	13.3	13.3	86.7	15.4	84.6
		Total %	26.0	4.0	4.0	26.0	7.7	42.3
Total		Number	26	24	27	23	9	17
		%	52.0	48.0	54.0	46.0	34.6	65.4
		Total %	52.0	48.0	54.0	46.0	34.6	65.4

Table 1 shows that Demodex was found in 13 (50%) of the patients in the study (Figure 1). The parasite incidence rate was significantly higher in the alcohol abuse group than the control group (p=0.002). The mean age of the patients was 44.69±9.785 year and the mean disease duration was 18.12± 11.476 years. The patient group consisted of 26 subjects and the control group of 24 subjects.

The demographic data of the subjects included in the study are presented in table 2.

As seen in table 2, the study group consisted of 46 males and 4 females. The alcohol abuse group included 24 (92.3%) males and 2 (7.7%) females for a total of 26 patients. Among this group, the number of smokers was 23 (88%) and the number of patients with a positive skin finding was 17 (65.4%).

The distribution of Demodex in the study groups is presented in table 3.

There was a significant relationship between Demodex and both alcohol abuse (p=0.001) and smoking (p<0.001).

Figure 1. *Demodex spp.* adult larva and egg (10X).

Discussion

The Demodex infestation rate has been reported not to differ according to race and gender and the parasite to be widespread globally (Rufli et al., 1981). The rate varies according to age in healthy individuals and reaches 100% in the elderly (Rufli et al., 1981). Various studies have also reported that infestation is not observed in children in the absence

of an immunosuppressive disorder and the incidence rate increases with age after puberty. (Karaman et al., 2014; Durmaz et al., 2015; Demirdag et al., 2016). Wang reported an infestation rate of 51.5% in 2248 patients aged 18-27 years in his 1848 study (Wang and Hu, 2001). The mean age of the patients in the current study was 44.69±9.785 years and Demodex was found in 13 (50%). The incidence rate was significantly higher in the patient group than the control group (p=0.002).

We used the SSSB method to diagnose the parasite and the samples were evaluated immediately. The Hoyer solution used in the study has been reported to provide a clearer view of the mites in relevant studies, making it suitable for diagnostic use (Tilki et al., 2017). The diagnostic method used in this study can therefore considered to be quite effective in detecting the parasites.

Various studies have reported that Demodex spp. can infest every hair follicle in several regions of the body. However, the parasite density can be higher in the facial region and cheek than in the other regions (Erbagci and Ozgoztasi, 1998; Karaman et al., 2014; Durmaz et al., 2015; Demirdag et al., 2016). We chose the facial region for the sample as it has the highest incidence rate and the patients allowed only one sample to be taken.The prevalence range in healthy individuals is 6.7%-74% for D. folliculorum, 11.1%-30.7% for D. brevis and 10.07%-74.7% for Demodex spp. (Tilki et al., 2017). The Demodex spp. incidence was found to be 8.3% in the control group in our study and this is similar to results from other studies.

Various studies on Demodex spp. incidence have evaluated Demodex epidemiology. A study from Sivas used SSSB and the eyelashes from 47 patients with chronic kidney failure and control subjects. D. folliculorum was found in the eyelash follicles of 6 patients and on the face of 12 patients. These rates were higher than found in the control group (Ozcelik et al., 2007). Inci et al. (2012) found D. folliculorum in 22.4% of the cancer patients and 3.2% of the control group with the SSSB method in 49 patients with urological cancer and 31 healthy controls. The parasite density in dialysis patients was found to be significantly higher than the control group in a study on 67 dialysis patients and 67 healthy individuals in Malatya (Karincaoglu et al., 2005). The infestation rate was 19.54% (Duzgun and Aytekin, 2007) in 87 hemodialysis patients in Diyarbakır, 28.9% in 45 patients receiving phototherapy. The rates were 12% in 41 patients with rheumatoid arthritis and 8% in 27 controls in another study from Afyonkarahisar (Ciftci et al., 2007) and 24.5% in 290 patients with various chronic diseases such as diabetes, renal failure, hypertension, hyperthyroid and cancer and 6% in 100 control subjects in Van (Tas et al., 2010). We found a Demodex positivity rate of 50% in this study.

Alcohol suppresses the immune system. Alcoholic pathology is known to impair the body's cytokine balance and function. Cell immunity is insufficient in moderate and heavy drinkers due to the direct toxic effect of alcohol on the bone marrow. The number and function of T cells are decreased in chronic addicts. Similarly, the number and function of granulocytes are also decreased, together with chemotaxis and phagocytosis. The humoral immune system is affected less than the cellular immune system. However, heavy drinkers experience shows a decreased primary antigen reaction to antibody production (Crews et al., 2006; Kockaya et al., 2016)

It has been reported that the immune status of the host can also influence the Demodex infestation rate. On the other hand, the number of mites living commensally in healthy individuals may increase as with opportunistic pathogens in diseases such as AIDS and malignancies that cause immune dysfunction (Jansen et al., 2001; Aquilina et al., 2002; Akilov and Mumcuoglu, 2004; Inci et al., 2012). Chovatiya et al. (2016) found an increased Demodex spp. prevalence in conditions weakening the immune system such as organ transplantation. Another study has reported that Demodex infestation can be more common in diabetic patients as the disease may increase the possibility of an immunosupressed state (Akdeniz et al., 2002). Disorders compromising the immune system such as cancer, chemotherapy, chronic renal failure, and organ transplantation have been reported to potentially cause D. folliculorum infestation (Bastemir et al., 2015). Similarly, Zeytun and Olmez (2017) found that immunosuppression can significantly increase Demodex density and that COAD patients have higher rates of Demodex infestation than control subjects. They reported that Demodex infestation can develop in patients receiving immunosuppressive treatment and that this should be taken into account. We found a positive association between Demodex presence and alcohol abuse (p=0.001) and smoking (p<0.001) in this study.

In conclusion, the Demodex spp. infestation incidence can increase in patients with alcohol abuse and should be considered. Patients with

alcohol abuse are occasionally admitted to psychiatric clinics for treatment. Nurses and ancillary health care staff should be careful in terms of erythema and papulopustular lesions and request a dermatology consultation when necessary. We suggest considering that Demodex species can be opportunistic pathogens especially in erythematous, itchy facial lesions in this patient group and providing the necessary treatment.

Ethics Committee Approval: Ethics committee approval was received for this study from Inonu Clinical Research Ethics Committee of InonuUniversity (Ethichs No: 2019-01-07).
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RESEARCH ARTICLE

The efficacy of Tc-99m DMSA scintigraphy in children with vesicoureteral reflux accompanying frequent urinary tract infection

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Abstract

Objective: Urinary tract infection is one of the most common diseases in childhood. Experimental studies have shown that renal damage due to urinary tract infection can be prevented by early diagnosis and treatment. The aim of this study was to evaluate the correlation between vesicoureteral reflux grade and Technetium-99m- dimercaptosuccinic acid scintigraphy in patients with frequent urinary tract infection

Methods Between January and October 2017, 34 cases were diagnosed as vescoureteral reflux with voiding cystoureterography were retrospectively evaluated with Technetium-99m- dimercaptosuccinic acid scintigraphy in terms of renal scar and function. Scars and function ratios were evaluated in Technetium-99m- dimercaptosuccinic acid scintigraphy according to voiding cystourethrography grades and correlation between the vesicoureteral reflux grade and Technetium-99m- dimercaptosuccinic acid scintigraphy findings were investigated.

Results: Of 34 patients, 26 (76.5%) were female and 8 (23.5%) were male. The mean age of the patients was 6.08 ± 4.03 (min: 1 month, max: 17 years). Twenty-one patients had unilateral vesicoureteral reflux and 13 patients had bilateral vesicoureteral reflux. Vesicoureteral reflux were detected in 47 of the total 68 kidneys.

Conclusion: In children with vesicoureteral reflux, Technetium-99m- dimercaptosuccinic acid scintigraphy provides useful information to show the kidney damage and as the vesicoureteral reflux grade increases, the rate of kidney damage increases. Even in the case of grade 1 and grade 2 vesicoureteral reflux, which are not expected to cause renal parenchymal damage. Technetium-99m- dimercaptosuccinic acid scintigraphy is the most successful imaging method for detecting parenchymal injury.

Key words: DMSA; Vesicoureteral Reflux; Voiding Cystoureterography

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Introduction

Urinary tract infection is one of the most common diseases in childhood. This infection can only be limited to the bladder (cystitis) or may result in renal parenchymal injury (pyelonephritis) (Tekgul et al., 2012). The risk of developing renal parenchymal injury has increased, especially in children with high degrees of vesicoureteral reflux (VUR) (Temiz et al., 2006). Therefore, if VUR is not excluded in children with frequent urinary tract infections (more than two in six months, three or more in one year), end stage renal failure may be developed in these patients. In all children who have a frequent urinary tract infection and the children who have a febrile urinary tract infection under 2 years of age, voiding cystourethrography (VCUG) is recommended. Experimental studies have shown that renal damage due to urinary tract infection can be prevented by early diagnosis and treatment (Hoberman et al., 2003). Therefore, detection of VUR and renal scar is of great importance. The sensitivity of Technetium-99m dimercaptosuccinic acid (Tc-99m DMSA) scintigraphy in detection acute and chronic pyelonephritis is higher than intravenous pyelography and ultrasonography (Stokland et al., 1999). In addition, it is considered the gold standard for the detection of renal scar (Bhatnagar et al., 2002; Piepsz et al., 1999). The aim of this study is to emphasize the importance of Tc-99m DMSA scintigraphy in evaluating the relationship between VUR grade and renal parenchymal injury in patients with VUR.

Methods

Design of the study

34 patients aged 0-18 years with vesicoureteral reflux were included in the study. These children who admitted to Hatay Mustafa Kemal University Pediatric Surgery Clinic between January and October 2017 were evaluated retrospectively. All patients presented with frequent urinary tract infection (more than two in six months, three or more in one year) and were diagnosed VUR via VCUG.

With the decision dated 11.10.2018 and numbered 12, ethics committee approval was obtained. Antibiotic prophylaxis was initiated in all patients diagnosed with VUR and Tc-99m DMSA scintigraphy was performed after there was no growth in urine cultures. Patient's VUR grades and also defected/hypoperfused areas and renal functions in Tc-99 mm DMSA scintigraphy were recorded.

Tc-99m DMSA scintigraphy capture

After obtaining informed consent from the parents of the patient, Tc-99m DMSA was administered intravenously at a dose of 1-5 mCi according to the age of the patient. Three hours later, the two-headed gamma camera (Siemens, Symbia S, Germany) and static images were taken at posterior, anterior and posterior oblique positions. Images were taken with low energy general purpose collimator in 256x256 matrix using 140 keV ± energy window. 1000 kkount count was taken for each position.

Scars and function ratios were evaluated in Tc-99m DMSA scintigraphy according to VCUG grades and correlation between the VUR grade and Tc-99m DMSA scintigraphy findings were investigated.

Statistical analysis

Data were analyzed using Statistical Pack for Social Science for Windows 18.0 version. (SPSS Inc., Chicago, IL, USA). Quantitative data were assessed were examined by student's t test. Qualitative data were evaluated by ki Chi-square test. $P < 0.05$ was considered significant.

Results

Of the 34 patients, 26 (76.5%) were female and 8 (23.5%) were male. The mean age of the patients was 6.08 ± 4.03 (min: 1 month, max: 17 years). Twenty-one patients had unilateral VUR and 13 patients had bilateral VUR. VUR was detected in 47 of the 68 kidneys in total (Table 1). There were 7 kidneys with grade 1, 5 kidneys grade 2, 16 kidneys with grade 3, 15 kidneys with grade 4, and 4 kidneys with grade 5 VUR (Table 2).

Table 1. Clinical features of patients

	n	%
Number of Patients	34	
Male	8	76.5
Female	26	23.5
Mean age all of the patients	6,08	
Male	5,4	
Female	6,28	
Side of vesicoureteral reflux		
Left	9	26,47
Right	12	35,29
Bilateral	13	38,23
Mean function (%)		
On the VUR side	35	
On the non VUR side	64,64	

Table 2. The number of kidneys according to vesicoureteral reflux grades

VUR Grade	The number of kidney (n=47)
Grade 1	7
Grade 2	5
Grade 3	16
Grade 4	15
Grade 5	4

Mean age of patients with grade 1 reflux was 4, patients with grade 2 reflux was 5, patients with grade 3 reflux was 7.25, patients with grade 4 reflux was 5.8, patients with gr5 reflux was 8. One patient with grade 5 reflux was 1 month old.

The mean function on the VUR side of 21 patients with unilateral VUR was 35.36 ± 16.33% and the mean function on the non-VUR side was 64.64 ± 16.33%. Of the 47 kidneys with VUR, 25 kidneys had cortical defect / hypoperfusion. While there is no pathology in 22 kidneys; perfusion defect were observed globally in 14 kidneys, on the upper pole in 4 kidneys, on the both upper and lower poles in 4 kidneys, on the lower pole in 2 kidneys and on the both upper pole and the middle section in 1 kidney (Table 3) (Figure 1).

Table 3. The number of kidneys with vesicoureteral reflux and perfusion defect; and also localization of defects

Defect localization	The number of kidney (n=47)
Normal (no defect)	22
Global	14
Upper pole	4
Upper and lower pole	4
Lower pole	2
Upper pole and central part	1

The incidence of perfusion defects was 14.3% in grade 1 VUR, 40% in grade 2 VUR, 43.7% in grade 3 VUR, 73.3% in grade 4 VUR and 100% in grade 5 VUR (Table 4). In low-grade VUR (Grade 1 and 2), the incidence of perfusion defects in the kidneys was 25%, while in the high-grade VUR (Grade 3 and above) was 62.86%. Scar function increases significantly as the degree of VUR increases. P:<0.28 (1 and 5)

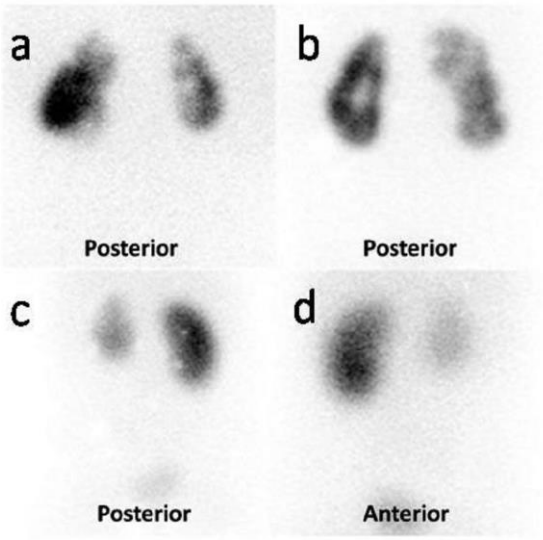

Figure 1. 9-year-old girl, multiple defects in both kidneys, more prominent on the right, function rates were 74% in left kidney, 26% in right kidney, and grade 4 VUR in bilateral kidneys (a), 2-year-old girl, multiple defects in both kidneys, more prominent on the right, function rates left kidney 51%, right kidney 49%, bilateral kidneys grade 4 VUR (b), 5-year-old girl, global hypoperfusion in the left kidney, function rates in the left kidney 17%, right kidney 83%, left kidney grade 3 VUR, posterior image (c), anterior view of the same patient (d).

Table 4. Rates of perfusion defects in kidneys according to vesicoureteral reflux grade

VUR Grade	Number of kidney with defect	Number of kidney without defect	Percentage of perfusion defects
Grade 1	1	6	%14,3
Grade 2	2	3	%40
Grade 3	7	9	%43,7
Grade 4	11	4	%73,3
Grade 5	4	0	%100

Discussion

Vesicoureteral reflux is the most common anomaly in pediatric urinary tract infections (Yılmaz et al., 2018). The rate of VUR diagnosis in children with frequent urinary tract infections has been reported to be 30-50% (Simsek et al., 1991). VUR is the most common risk factor in the development of renal damage (Camacho et al., 2004), resulting in renal parenchymal damage as well as proteinuria, hypertension and end-stage renal failure in children (Peters et al., 2010).

Diagnosis, treatment and follow-up of these patients are very important and children with frequently urinary tract infection should be investigated for VUR. Because, if the early diagnosis of VUR can be made and treated, the renal function of these patients can be preserved.

The presence of renal scarring is a most critical determinant factor in the surgical treatment of VUR. Intravenous pyelography (IVP) was the most commonly used method for evaluating renal parenchyma before radionuclide imaging methods developed. In low grade VUR's where pelvicaliseal dilatation is not observed, IVP can be mostly normal. In addition, it was reported that 6 to 24 month period was required for renal damage to be seen in the IVP. Renal damage can be detected much earlier via Tc-99m DMSA scintigraphy (Rushton et al., 1992). Ultrasonography is the most commonly used imaging method for this purpose because of its cheap, easily accessible and no radiation. However, Tc-99m DMSA scintigraphy is much more sensitive in detecting parenchymal defects, İt has been reported that USG cannot detect 40% of scars and these renal scars can be detected by Tc-99m DMSA scintigraphy (Temiz et al., 2006). Therefore, Tc-99m DMSA scintigraphy has been accepted as the gold standard in detecting the presence of renal scar (Roebuck et al., 1999).

The indications of Tc-99m DMSA scintigraphy is detection of renal parenchymal abnormalities, renal sequelae (six months after infection), acute pyelonephritis, associated abnormalities (abnormal duplex kidney, small kidney, dysplastic tissue, horseshoe kidney), ectopic kidney and confirmation of non-functional multicystic kidney. There are no contraindications for Tc-99m DMSA scintigraphy. During the acquisition, immobilization of the child should be obtained. The most difficult age is between 1-3 years, but sedation is rarely needed. Tc-99m MAG3 scintigraphy can also be used to detect renal parenchymal defects, but Tc-99m MAG3 scintigraphy images are acquired only in posterior or anterior and posterior positions without oblique positions. You do not have a second chance to take the images again with Tc-99m MAG3 scintigraphy, because it is a dynamic acquisition. But in Tc-99m DMSA scintigraphy, the technician can acquire the images again in case of any problem such as patient movement.

In case of significant hydronephrosis late Tc-99m DMSA images (4 to 24 hours) or diuretic injection may be useful to excrete Tc-99m DMSA activity from the renal pelvis. In these cases Tc-99m MAG3 scintigraphy should be considered (Piepsz et al., 2009).

It can detect acute pyelonephritis if it is used during the acute illness, but it shows renal scars secondary to acute disease in late imaging performed months after infection (Wani et al., 2016). In Tc-99m DMSA scintigraphy, wedge-shaped cortical defects and decreased renal volume associated with decreased cortical function are significant for determining renal scarring (Agras et al., 2007). In a study recommended that all children with recurrent urinary tract infections should be routinely investigated for VUR via VCUG in addition to USG and DMSA scintigraphy should be performed in non-acute period (Doğan et al., 2018). Wongbencharat et al found that the sensitivity of urinary tract infection was 50% and Tc-99m DMSA scintigraphy in the sixth month was 87.5% in predicting infants with high grade VUR (Wongbencharat et al.,2016).

Awais et al reported that Tc-99m DMSA scintigraphy has high sensitivity and negative predictive value on patients with high grade VUR (Grade 3 and higher) and also it has benefit of exclusion of high grade VUR (Awais et al., 2015). In our study, as the VUR grade increased, the probability of renal damage in the kidneys was increased. The incidence of perfusion defect in low grade VUR was 25%, whereas it was 62.86% in high-grade VUR. Urinary tract infection accompanied fever do not cause renal damage in most children, even if the resulting damage it is generally remains unchanged or recedes over time. However, 20% of children with kidney damage after urinary tract infection have impaired renal function. Patients with impaired renal function are usually children with grade 3-5 VUR (Swerkersson et al., 2017). There is a direct correlation between renal scar development and VUR grade (Temiz et al., 2006). In our study, the frequency of perfusion defect / scar development in the kidneys increased as the VUR degree increased. An important point was that no pathology was found in 22 (46.80%) of 47 kidneys with VUR. An important point was that no pathology was found in 22 (46.80%) of 47 kidneys with VUR. This result supports the hypothesis that if there is no observed pathology in the TC-99m scintigraphy in early period, the risk of developing renal scar is low (Biggi et al., 2001; Stockland et al., 1996).

Conclusion

Vesicoureteral reflux is an important cause of chronic renal failure in children if it is not diagnosed

and treated in time. The severity of the renal parenchymal injury should be evaluated after the diagnosis of VUR. In children with VUR, Tc-99m DMSA scintigraphy provides useful information to show the renal damage and as the VUR grade increases, the rate of renal damage increases. Even in the case of grade 1 and grade 2 VUR, which are not expected to cause renal parenchymal damage. Tc-99m DMSA scintigraphy is the most successful imaging method for detecting parenchymal injury, which can detect early damage with less radiation exposure.

Ethics Committee Approval: Ethics committee approval was received for this study from Clinical Research Ethics Committee of Hatay Mustafa Kemal University (2018-12).
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RESEARCH ARTICLE

Determination of *Trichomonas vaginalis* Positivity and Risk Factors in Patients with Urogenital Complaints

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Abstract

Objective: In this study, the incidence and prevalence of *Trichomonas vaginalis* was aimed to help to overcome the difficulties in explaining the data for the regions and it was aimed to investigate its prevalence in different social groups in Ordu and its environs.

Methods Trichomonas Questionnaire Form and Personal Information Form of *T. vaginalis* were used in those who came to the urology and gynecology outpatient clinics from Ordu province and its environs. Urine sediment from the patients of urology outpatient clinic and vaginal smear specimen from the patients of gynecology outpatient clinic were collected and analyzed. All samples were examined by Direct Microscopic Investigation, culture, Giemsa and Papanikolau (PAPS) stain. Statistical analysis was performed with one-way and two-way chi-square test.

Results: In this study, 713 patients (202 males and 511 females) were examined. A total of 83 patients (11.6%) were positive. There was a difference in the frequency distribution of the individuals with positive *T. vaginalis* with respect to age, gender, marital status, economic status, educational status, settlement, working status and home living status, knowledge status about infectious diseases, going to the bath, going to the pool, traveling, type of toilet, use of toilet paper, vaginal discharge, vaginal discharge status, drug usage, abortion, and knowledge status about sexually transmitted diseases and *T. vaginalis* ($p < 0.001$).

Conclusion: In this study, it was concluded that marital status, economic status, education level, settlement, working status and travel frequency may be effective in transmission of parasite. In this respect, it was inferred that the transmission of *T. vaginalis* can be reduced with in-service and public health trainings on the parasite transmission and the ways of prevention

Key words: *Trichomonas vaginalis*, urine, vaginal smear, urogenital,

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Introduction

Trichomonas vaginalis is a flagellated protozoan that lives in the human urogenital system. There is only trophozoite form of it. The disease caused by it in the human urogenital system is called Trichomoniasis.

Trichomoniasis is a common infection all over the world. The rate of infection varies greatly from country to country, from community to community. The incidence is very high, especially in societies where women lack sexual hygiene precautions. According to the literature data, the incidence of Trichomoniasis in women varies between 10 and 90%. Infection rate is reported as 9% in males (Unat et al; 1995). Due to the reasons like the use of different techniques and incomplete evaluation of known techniques, discrepancies between literature data are noteworthy in terms of the incidence of urogenital Trichomoniasis (Budak, 1987; Kuman, 1996; Cetinkaya et al, 2011). Although Trichomoniosis is a common infection, it has been reported that it is not possible to determine its incidence, and researchers have attributed the reason for the great variation in the rate of infection from country to country and from society to society to the fact that the methods used in diagnosis and the selected human populations are different (Toker, 1995; Daldal et al, 2002, Aral Akarasu, 2006). The direct transmission method of the parasite is the sexual route, but indirect transmission can also be observed (Karaman et al, 2006).

In the studies performed on the epidemiology of *Trichomonas vaginalis* in in Turkey, it is not possible to precisely state the incidence of this parasite due to the fact that it is locally limited (Kuman, 1996).

In this study, the incidence and prevalence of *Trichomonas vaginalis* in Turkey was aimed to help to overcome the difficulties in explaining the data for the regions and it was aimed to investigate its prevalence in different social groups in Ordu and its environs. In addition, it is aimed to gain the habit of confirming the diagnosis by using diagnostic methods, to increase the habit of using direct examination and culture methods, and to determine the prevalence of Trichomoniasis, to provide treatment and to define the risk factors of Trichomoniasis in patients with the complaint.

Materials and Methods

Material Collection and Performing Questionnaire

Trichomonas Questionnaire Form which was developed in order to measure the prevalence of *T. vaginalis* with respect to various social conditions in the patients coming to the urology and gynecology outpatient clinics from Ordu province and its environs -the dependent variable of the study- and Personal Information Form again for the independent variables of the personal information in the study were used. The data of the study was collected by using face to face interview method between 10 June 2016-10 June 2017. Filling of data collection forms and obtaining samples took approximately 20-25 minutes. Male and female patients who agreed to participate in the study were asked to fill in the personal information form and trichomonas questionnaire form after signing the informed consent form. Illiterate individuals were asked to respond to forms after being read them. Literate participants answered the forms themselves. Samples were taken from the patients who participated in the study after the form was answered.

The social variables for Trichomoniosis examined in the study are limited to the characteristics measured by the Trichomonas questionnaire. It was assumed that women and men participating in the survey answered the questionnaire given to them sincerely and honestly and the questionnaire prepared was of sufficient validity and reliability.

The data collection process of the study was carried out in the urology and gynecology policlinics in Ministry of Health Ordu University Training and Research Hospital. The analysis process was realized in Parasitology Laboratory and Histology and Cytology Laboratory of Basic Sciences Department at Morphology Building of Faculty of Medicine, Ordu University.

Methods Applied

For parasite diagnosis, urine sediment from the patients administered to urology outpatient clinic and vaginal smear specimen from the patients administered to gynecology outpatient clinic were collected and analyzed. All samples were examined by Direct Microscopic Investigation, and Giemsa and Papanikolau (PAPS) stain. In addition, each sample was cultured on Cysteine-Peptone-Liver-Maltose (CPLM) medium and evaluated after two days.

Biostatistics Analysis

Data was summarized in numbers and percentages. One-way and two-way chi-square test

were used to statistical analysis of categorical variables. The statistical significance levels were set at ($p<0.05$). All statistical analyses were performed using IBM SPSS Statistics for Windows, version 26.0 (IBM, Armonk, NY, USA).

Results

In this study, 713 patients (202 males and 511 females) were examined. A total of 83 patients (11.6%) were positive. 83 positive cases were detected by direct examination, staining method and culture method used in the diagnosis of *T. vaginalis* (Tablo 1). There were 79 positivity by direct microscopy, 81 positivity by staining method and 83 positivity by culture method.

The incidence of *T. vaginalis* was detected as 14.7% in women and 4% in men (Tablo 2).

The distribution and comparison of some characteristics of the study group according to *T. vaginalis* results were given in Table 3.

When Table 3 was examined, it was found that there was a difference in the frequency distribution of age, gender, marital status, economic status, educational status, settlement, working status, home living status and knowledge status about infectious diseases groups in the individuals with positive *T. vaginalis*. In addition, the incidence of parasitic diseases increased as infectious diseases information status decreased ($p <0.001$). Again, the incidence of it in married was higher than single and widows ($p<0.001$). The presence of *T. vaginalis* showed significant changes with gender, working status and home living status ($p <0.05$). In the study, higher positivity was observed in women compared to men. The analysis of the risk factors for *T. vaginalis* of the study group is given in Table 4a and 4b.

When Table 4a is analyzed, there was a significant difference in frequency distribution of *T. vaginalis* positive individuals with respect to going to hammam, going to pool, travel, type of toilet, toilet paper usage and discharge ($p <0.001$). The presence of *T. vaginalis* showed a significant change in terms of going to swimming pool, toilet type and the smell of discharge ($p <0.05$).

When Table 4b is analyzed, there was a significant difference in frequency distribution of *T. vaginalis* positive individuals with respect to discharge status, drug usage, abortion, and knowledge level about sexually transmitted diseases and *T. vaginalis* ($p <0.001$). The presence of *T. vaginalis* showed a significant change in terms of having children and abortion status ($p <0.05$).

Table 1. *T. vaginalis* Distribution

Presence status of *T. vaginalis*	n	%
Negative	630	88.4
Positive	83	11.6
Total	713	100

Table 2. *T. vaginalis* Distribution with respect to Gender

Gender	Presence status of *T. vaginalis*				Total	
	Negative		Positive			
	n	%	n	%	n	%
Woman	436	85.3	75	14.7	511	71.7
Man	194	96.0	8	4.0	202	28.3
Total	630	92.1	83	7.9	713	100

Table 3. The distribution of *T. vaginalis* positivity of the study group according to demographic characteristics

		Trichomonas vaginalis					
		Positive		p^1	Negative		p^2
		n	**%**		n	%	
Age	0-19	1	12.5	<0.001	7	87.5	0.529
	20-34	20	12.4		141	87.6	
	35-50	36	13.4		232	86.6	
	>50	26	9.5		249	90.5	
Gender	Woman	75	14.7	<0.001	436	85.3	<0.001
	Man	8	4.0		194	96.0	
Marital Status	Single	3	6.5	<0.001	43	93.5	0.397
	Married	76	12.3		544	87.7	
	Widow	4	8.5		43	91.5	
Economic Status	Good	10	11.8	<0.001	75	88.2	0.116
	Medium	69	12.7		476	87.3	
	Low	4	4.8		79	95.2	
Educational Status	Illiterate	12	17.4	<0.001	57	82.6	0.085
	Primary education	50	13.2		329	86.8	
	High school	15	8.2		168	91.8	
	Higher education	6	7.4		75	92.6	
Education Status of Spouse	No spouse	7	7.7	<0.001	84	92.3	0.268
	Illiterate	2	4.8		40	95.2	
	Primary education	50	13.7		314	86.3	
	High school	18	11.8		134	88.2	
	Higher education	6	9.4		58	90.6	
Settlement	Village	19	13.1	0.001**	126	86.9	0.580
	City	20	9.8		185	90.2	
	Province	44	12.1		319	87.9	
Working Condition	Unemployed	69	15.5	<0.001	376	84.5	<0.001
	Worker / officer / retired	14	5.2		254	94.8	
	Officer	0	0.0		0	0.0	
	Retired	0	0.0		0	0.0	
Working Condition of Spouse	No spouse	7	7.6	<0.001	85	92.4	0.395
	Unemployed	30	11.6		229	88.4	
	Worker / officer / retired	46	12.7		316	87.3	
Home Living Status	Alone	1	2.9	<0.001	34	97.1	0.008**
	Extended family	5	4.6		103	95.4	
	Nuclear family	77	13.5		493	86.5	
Another Woman if Extended Family	Not extended family	65	15.7	<0.001	348	84.3	<0.001
	Yes	8	11.9		59	88.1	
	No	10	4.3		223	95.7	
Information Status about Infectious Diseases	Good	11	8.0	0.001**	126	92.0	0.114
	Medium	16	9.7		149	90.3	
	Low	21	17.1		102	82.9	
	Absent	35	12.2		253	87.8	

p^1:One-way chi-square test, p^2:two-way chi-square test

* *p<0.05*

** *p<0.01*

Table 4a. The analysis of the risk factors of the study group with respect to *T. vaginalis*

Variables		*Trichomonas vaginalis*					p[2]
		Positive			Negative		
		n	%	p[1]	n	%	
Do you go to the swimming pool?	Always	0	0.0	<0.001	3	100.0	0.031*
	Sometimes	3	3.4		84	96.6	
	Never	80	12.8		543	87.2	
Do you go to the hammam?	Always	0	0.0	<0.001	7	100.0	0.577
	Sometimes	12	10.6		101	89.4	
	Never	71	12.0		522	88.0	
Do you travel?	Always	0	0.0	0.062	38	100.0	0.006**
	Sometimes	50	10.5		424	89.5	
	Never	33	16.5		167	83.5	
Does your spouse travel?	No spouse	7	8.1	<0.001	79	91.9	0.289
	Always	1	3.2		30	96.8	
	Sometimes	52	12.8		353	87.2	
	Never	23	12.0		168	88.0	
What type of toilet do you use at home?	Squatting toilet	30	15.8	0.113	160	84.2	0.094
	Flush toilet	19	11.5		146	88.5	
	Both	34	9.5		323	90.5	
Do you use toilet paper?	Yes	70	11.4	<0.001	542	88.6	0.239
	No	10	17.5		47	82.5	
	Sometimes	3	7.0		40	93.0	
Do you have vaginal discharge symptoms?	Always	18	14.1	0.001**	110	85.9	0.067
	Sometimes	43	13.7		270	86.3	
	Never	22	8.1		250	91.9	
If you have vaginal discharge, how long has it been?	No vaginal discharge	22	8.4	<0.001	241	91.6	0.091
	1. week	10	13.7		63	86.3	
	2. week	10	16.7		50	83.3	
	3. week	0	0.0		25	100.0	
	4. week	4	12.9		27	87.1	
	5. week	37	14.2		224	85.8	
If you have vaginal discharge, does it smell?	No vaginal discharge	22	8.4	0.201	241	91.6	0.028*
	Yes	35	16.2		181	83.8	
	No	26	11.1		208	88.9	
If you have vaginal discharge, what color is it generally?	No vaginal discharge	22	8.3	<0.001	242	91.7	0.265
	White	26	11.9		192	88.1	
	Yellow	31	15.2		173	84.8	
	Green	2	11.8		15	88.2	
	Red	2	22.2		7	77.8	
	Black	0	0.0		1	100.0	
Do you use medicine for your vaginal discharge?	No vaginal discharge	22	8.3	<0.001	242	91.7	0.128
	Yes	11	17.5		52	82.5	
	No	50	13.0		335	87.0	
	Sometimes	0	0.0		1	100.0	
Did you apply to a health facility when your vaginal discharge symptoms started?	No vaginal discharge	22	8.3	0.091	242	91.7	0.064
	Yes	24	11.9		178	88.1	
	No	37	15.0		210	85.0	

Table 4b. The distribution of *Trichomonas vaginalis* positivity according to study questions

Sorular		*Trichomonas vaginalis*					p[2]
		Positive			Negative		
		n	%	p[1]	n	%	
Do vaginal discharge symptoms affect your sexual life?	No vaginal discharge	0	0.0	<0.001	3	100.0	0.229
	Frequently	7	13.2		46	86.8	
	Sometimes	22	16.9		108	83.1	
	No effect	48	10.7		401	89.3	
	No sexual life	6	7.7		72	92.3	
Do you have pain during sexual intercourse?	No spouse	6	7.5	<0.001	74	92.5	0.216
	Frequently	10	18.2		45	81.8	
	Sometimes	24	13.4		155	86.6	
	No	43	10.8		356	89.2	
What is the frequency of sexual intercourse?	No spouse	7	8.6	<0.001	74	91.4	0.356
	Once in 1-2 days	3	5.4		53	94.6	
	Once in 3-4 days	27	15.2		151	84.8	
	Once in 5-6 days	16	12.4		113	87.6	
	Once in 7 days and more	30	11.3		236	88.7	
	No sexual intercourse with spouse	0	0.0		3	100.0	
What is the frequency of changing your underwear?	Once in 1-2 days	66	12.0	<0.001	482	88.0	0.525
	Once in 3-4 days	13	9.4		126	90.6	
	Once in 5-6 days	4	18.2		18	81.8	
	Once in 7 days and more	0	0.0		4	100.0	
Do you have children?	Yes	78	12.8	<0.001	532	87.2	0.020*
	No	5	4.9		98	95.1	
Have you ever had an abortion?	Patient man	7	3.5	<0.001	191	96.5	<0.001
	Yes	29	14.3		174	85.7	
	No	47	15.1		265	84.9	
Do you use one of the birth control methods?	Yes	29	15.4	0.006**	159	84.6	0.059
	No	54	10.3		471	89.7	
What is your knowledge level on sexually transmitted diseases?	Very good level	3	6.5	<0.001	43	93.5	0.478
	Good level	11	9.2		108	90.8	
	Low level	25	13.4		162	86.6	
	I have no information	44	12.2		317	87.8	
What is your your knowledge level about the disease called “Trichomoniosis??	Very good level	0	0.0	<0.001	6	100.0	0.746
	Good level	2	12.5		14	87.5	
	Low level	3	8.3		33	91.7	
	I have no information	78	11.9		577	88.1	

p[1]:One-way chi-square test, p[2]:two-way chi-square test
* *p<0.05*
** *p<0.01*

Discussion

T. vaginalis was found to be positive at different rates according to the study area, living conditions, and the population of the epidemiological study. According to the studies performed in different countries found in literature data, *T. vaginalis* was detected at rates of 9% (Budak,1987) and 6% (Daviez and Clay, 1992) in England, and %3,2 in Sivas (Selvioglu et al, 2006), 6% (Acholonu and Walker, 1998), 7% (Madico et al.,1998) and 9% (Paterson et al., 1998) in USA, 10% (Vishwanath et al., 2000) and 7% (Sharma et al.,1991) in India. In the studies conducted in different regions of Turkey, *T. vaginalis* has been reported at the rate of 7% (Budak, 1987) and 9% (Sapmaz, 1985) in İzmir and its environs, 8% (Kilimcioglu et al.,1998) in Manisa and its environs, 8% (Ay and Yilmaz, 1994; Degerli at al., 1997) in Elazığ and its environs, 9% (Sadr et al., 1992) in Adana, 9% (Ay at al.,1996) Bursa, 7% (Toker, 1995) in Ankara and its environs, 10% (Turhanoglu et al, 1994) in Diyarbakır and 10% (Dogan and Aygun, 1999) in Eskişehir. Again, Karaman et al. (2006) detected the parasite at a rate of 8.1% in a study performed upon women in

Malatya. Similarly, Daldal et al. (2002), detected parasite in 14 of 33 bar girls working in the same region. In this study, 713 patients (202 males, 511 female) were examined. In total, parasites were detected in 83 (11.6%) patients. In the present study, a significant association was observed between the percentage of parasite incidence and gender. When Table 2 is examined, it is observed that the incidence of parasites is higher in females than males. When the results of the study were evaluated, it was concluded that the incidence of *T. vaginalis* varies according to the social structure of the populations, the selected population and the time of study.

Staining and culture methods are preferred in the diagnosis of parasite (Ertabaklar et al., 2004; Culha et al., 2006; Field et al., 2016; Akyildiz et al., 2018). Değerli ve ark (2011) 1.9% were positive with direct. In the present study, direct investigation, staining and culture methods were performed in parallel and similar results were obtained with each investigation.

The presence of *T. vaginalis* showed significant changes with gender, working status and home living status ($p<0.05$). In the study, higher positivity was observed in women compared to men. A significant increase was observed in the unemployed, according to both the presence or absence of parasites and the analysis among the positives. This situation can be explained that the epidemiology of the parasite may change with the socio-economic situation. Again, the percentage of incidence in the nuclear family was found to be higher. The aim of asking this question was thought that there may be indirect transmission since there may be more than one woman in the extended family. However, the rate of extended family found very low in the answers given to the questionnaire questions. This may be explained by the fact that the family status may be effective in the epidemiology of parasites.

It was found that there was a difference in the frequency distribution of age, gender, marital status, economic status, educational status, settlement, working status, home living status and knowledge status about infectious diseases groups in the individuals with positive *T. vaginalis*. In addition, the incidence of parasitic diseases increased as infectious diseases information status decreased.

The incidence of it in married women was higher than those of single and widows ($p<0.001$). This situation can be explained that active sexual life, economic status, education level, settlement and work status can be effective in parasite transmission.

When the responses to the questionnaire and the presence of the parasite were compared, there was no significant association with respect to frequency of going to hammam, renting or borrowing swimwear status, travel frequency of spouse, toilet paper usage status, bathing status, bathing style, the frequency of changing underwear, vaginal discharge complaint, color of the discharge, duration of vaginal discharge, disturbance status and pad usage status. However, a significant relationship was found in terms of the frequency of going to the swimming pool, the frequency of travel, the type of toilet in the workplace and the smell of the vaginal discharge. It has been reported that *T. vaginalis* can be transmitted via crowded pools and hammam (Unat et al, 1995; Sonmez Tamer, 2009). However, according to the questionnaire, the incidence of those who never went was high. In this respect, it has not been concluded whether going to the pool and hammam can be effective in the transmission or not. A significant relationship was found with respect to the type of toilet used, but it was not concluded whether the toilet type could be effective in the transmission or not since the incidence rate was high in unemployed ones. It has been reported that the parasite will be an odorous discharge (Cetinkaya et al., 2011) and the rate of being an odorous vaginal discharge in the presence of the parasite is high. This may be explained by the presence of odorous vaginal discharge, which may be suspected of the presence of the parasite. In the study, there was a significant relationship between the occurrence of the disease and the status of the out-of-drug treatment option ($p= 0.006$). The percentage of *T. vaginalis* is higher in those who do not try drugs. This can be interpreted as the patients do not use any medication other than the doctor's control.

According to the answers given to the questionnaire in the study, there was a significant difference in frequency distributions of the groups of going to the swimming pool and hammam, renting or borrowing swimwear usage, travel frequency, type of workplace toilet, toilet paper usage, discharge complaints, frequency of discharge, color of discharge, pad usage, itching complaints, used drugs, bath status, status of child and abortion, knowledge status on sexually transmitted diseases and trichomoniasis in *T. vaginalis* positive individuals. In the study, the rate of parasite incidence was found to be higher in those using toilet paper, having occasional complaints of

discharge, having vaginal discharge for 5 weeks or more and having white discharge. This can be explained by the fact that these factors may be effective in transmission of the parasite. It is reported in the literature data that the color of the discharge may be green (Cetinkaya et al., 2011), but in the present study, the percentage of parasite incidence was higher in those with white discharge. This may be due to the questionnaire questions and perceptions of the patients.

Conclusion

In this study, it was concluded that marital status, economic status, education level, settlement, working status and frequency of travel may be effective in transmission of parasite. Moreover, the fact that the incidence of parasites increases as the level of knowledge about sexually transmitted diseases and trichomoniosis decreases suggests that awareness studies should be performed. In this respect, it was concluded that the transmission of *T. vaginalis* can be reduced with in-service and public health trainings on the parasite transmission and the ways of prevention.

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RESEARCH ARTICLE

Is There an Association Between Pituitary Adenomas and Autonomic Cephalgia?

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Abstract

Objective: Trigeminal autonomic cephalalgias (TACs) are a group of primary headache syndromes. Although hemicrania continua (HC)is not included among the TACs according to the International Classification of Headache Disorders (ICHD-II), it also shows autonomic symptoms. In the literature, some authors suggest that TACs can be related with pituitary lesions. In this study, we have compared the pituitary lesions observed in the TAC, HC and migraine-type headache patients

Methods The cranial magnetic resonance images (MRI) of the patients with TAC, HC and migraine without aura (MWOA) unaccompanied by autonomic symptoms were retrospectively evaluated. The 33 TAC and HC patients were compared with the 30 migraine without aura (MWOA) patients.

Results: The mean age of the TAC and HC patients was 43. 36±10. 72 years, whereas the mean age of the MWOA patients was 39.0±6.92 years. No statistically significant difference in terms of age was observed between the groups (p>0.05). The MRI of the TACs and HC patients indicated that 62.5% (n:20) were normal, while 12.5% (n:4) had pituitary microadenomas, 6.3 % (n:2) had macroadenomas, 12.5% (n:4) had suspected pituitary microadenomas, and 6. 3% (n:2) had suspected heterogeneity. The MRI results of the MWOA patients were normal in 46.7% (n:14), whereas 23.3% (n:7) had pituitary microadenomas, 23.3% (n:7) had suspected pituitary microadenomas, and 6. 2% (n:2) had suspected pituitary heterogeneity. No statistically significant difference was observed between both groups (p>0. 05).

Conclusion: In this study, no correlation was observed between the TACs, HC and pituitary abnormalities. In conclusion, the use of pituitary MRI has not been supported as a routine method in patients with this type of headache

Key words: Hypophysis, Headache, Pituitary Hormones

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Introduction

Headaches are among the most commonly observed neurological symptoms and they constitute an important healthcare issue (Inan, 2011). Among the patients who present to neurology clinics, 2/3 complain from headaches and 1/3 present solely due to headaches (Baykan et al., 2011). According to the diagnostic criteria revised by the IHS in 2004, headaches have been classified in two main groups as primary and secondary headaches (Olesen, 2004). Primary headaches are more commonly observed. On the other hand, trigeminal autonomic cephalalgia, hemicrania continua (TOC-HC) belong to a less frequently observed group of primary headache syndromes characterized by unilateral headaches. As the name suggests, these headaches are associated with ipsilateral cranial autonomic findings and include cluster headaches, paroxysmal hemicranias, and short-lasting unilateral neuralgiform headache with unilateral conjunctival injection and tearing (SUNCT). Also, hemicrania continua is among the other primary headaches accompanied by autonomic symptoms (Saip, 2011). These autonomic symptoms can be observed with considerable frequency in case of headaches such as migraines (Uluduz, et al,2016)

The pathophysiology of autonomic headaches is yet to be cleared. In these types of headaches, secondary causes should be ruled out (Saip, 2011).

The secondary causes in the literature include a significant number of pathologies associated with the pituitary gland. However, among the cases reported in the literature, atypical features generally accompany headaches and therefore pituitary anomalies may be observed more frequently in these patients (Lai, 2009), (Elisabetta, 2009). The association of autonomic symptoms with pituitary adenomas has been studied before. The aim of the present study is to compare the pituitary magnetic rezonans image (MRI) anomalies of the patients with migraines unaccompanied by autonomic symptoms and the pituitary MRI anomalies in the patients with trigeminal autonomic headache and hemicrania continua. We also aim to investigate the requirement for pituitary MRI in the patients with headaches with autonomic symptoms and without atypical features.

Methods

Patients followed up by the Headache Outpatient Clinic of the Ministry of Health, Ankara Training and Research Hospital, Department of Neurology and diagnosed with TOC-HC and migraine without aura unaccompanied by autonomic symptoms based on the diagnostic criteria published by the International Headache Society (IHS) in 2004 (Baykan, 2011). were enrolled in the study. The study included 33 patients with TOC-HC, and 33 patients with migraine without aura unaccompanied by autonomic symptoms, of which the complete patient files could be retrieved.

The diagnoses of all the patients were reviewed based on the headache study forms. Their physical and neurological examinations were conducted by the same neurologist and the results were observed to be normal in all the patients. Patients with anomalies were excluded from the study. Patients with migraine or TOC-HC whose files contained brain MRI and pituitary MRI examinations were included in our study. The pituitary hormone test results of the patients in which the pituitary hormones were tested were noted. Brain MRI and pituitary MRI examinations were evaluated with specialist radiology and neurology.

The approval of the local ethics committee of Ministry of Health, Ankara Training and Research Hospital was obtained (2011) and the study was planned according to the latest version of the Helsinki Declaration and the Good Clinical Practices Guidelines.

Statistical Analysis

The study data were analyzed using the SPSS for Windows 11.5 software packages. The normality of the distribution of the continuous variables was investigated with the help of the Shapiro Wilk test. The descriptive statistics were indicated in mean ± standard deviation for the continuous variables and in patient number and percentage (%) for the nominal variables. The groups were compared using the suitable test from amongst the Chi-square test, Student's t-test or Fisher's Exact Test.

Statistical significance of the results was based on a value of $p<0.05$.

Results

For the purpose of our study, 40 patients with TOC-HCand 33 patients with migraine without aura unaccompanied by autonomic symptoms were enrolled in our study. However, since the initial statistical analysis pointed to an age difference, 7 patients from the TOC-HC group and 3 patients from the migraine group were excluded from the study and the statistical analysis was repeated. Consequently, our study included 33 patients diagnosed with TOC-HC and 33 patients with migraine without aura unaccompanied by

autonomic symptoms. The mean age of the patient group with TACs was 43.36 ± 10. 72, while the mean age of the patient group was 39. 0 ± 6.92. There was no statistically significant difference between both groups (p>0. 05). Among the TOC-HC patients, 45. 5% (n=15) were male while 54. 5% (n=18) were female. Among the migraine patients, 20% (n=6) were male while 80% (n=24) were female.

Among the patient group with TOC, 15.2% (n=5) were followed up for episodic cluster headaches, 9.1% (n=3) for chronic cluster headaches, 6.1% (n=2) for episodic paroxysmal hemicrania, 36.3% (n=12) for chronic paroxysmal hemicrania, 3% (n=1) for SUNCT, 27.3% (n=9) for hemicrania continua, and 3% (n=1) were followed up for stabbing headaches (Figure 1).

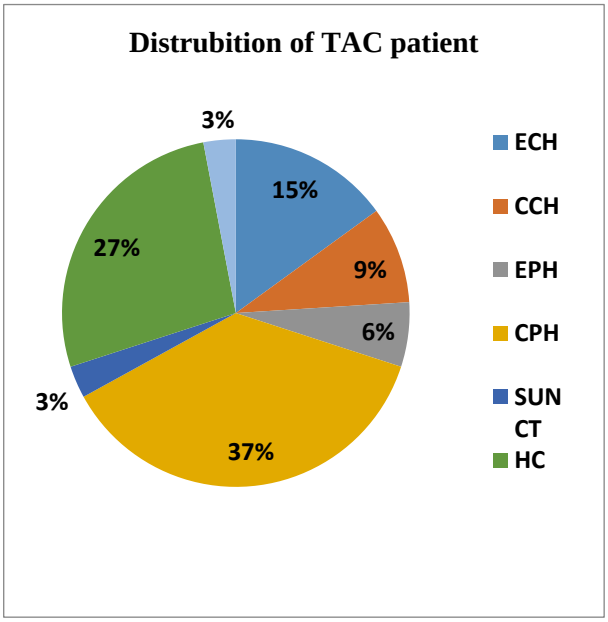

Figure 1. Distribution of TAC cases

The physical examination was normal in all the patients. No abnormalities were observed in the neurological examinations.

In the patient group with TOC-HC, 87. 9% (n=29) has unilateral headache which did not change sides, while this ratio was 26.7% in the group with migraine (n=8). The difference between both groups was statistically significant (p<0. 05).

The autonomic symptoms of the patients with TOC and HC included conjunctival injection and tearing in 48.5% (n=16), nasal discharge and nasal obstruction in 24.2% (n=8), conjunctival injection and tearing in 12. 1% (n=4), nasal obstruction and facial sweating in 6.1% (n=2), conjunctival injection and nasal obstruction in 6.1% (n=2), conjunctival injection and tearing and nasal discharge in 6.1% (n=2) and sweating in the forehead and face in 3% (n=1) (Figure 2).

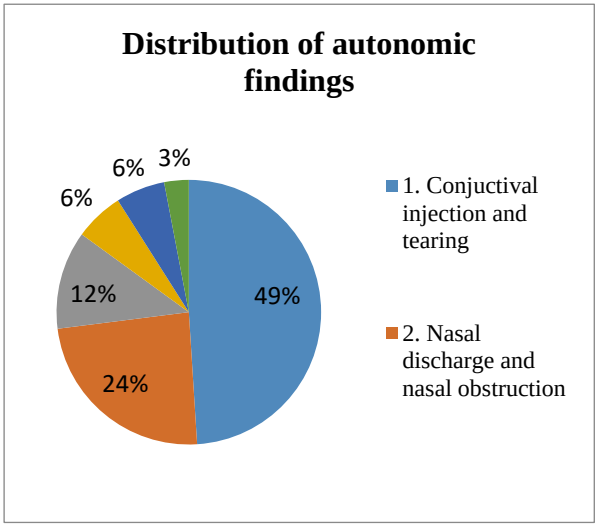

Figure 2. Distribution of autonomicfindings

Among the patients with TOC-HC, the pituitary MRI results were normal in 62.5% (n=20), while it revealed microadenomas in 12.5% (n=4), macroadenomas in 6.3% (n=2), suspected microadenomas in 12.5% (n=4), and suspicious heterogeneity in the pituitary gland in 6.3% (n=2). No cavernous sinus invasions were observed in any of the patients. The pituitary MRI of the patients with migraine were normal in 46.7 (n=14), while it revealed microadenomas in 23.3% (n=7), suspected microadenomas in 23.3% (n=7), and suspicious heterogeneity in the pituitary gland in %6,2 (n=2). No statistically significant difference was observed between the TOC-HC and migraine patient groups in terms of the occurrence and distribution of pituitary lesions (p>0. 05) (Figure 3).

Among the 33 TOC-HC patients, 25 could be reached, while 30 of the migraine patients could be contacted. No statistically significant difference was observed regarding the FSH, LH, ACTH, GH, PRL values (p>0. 05).

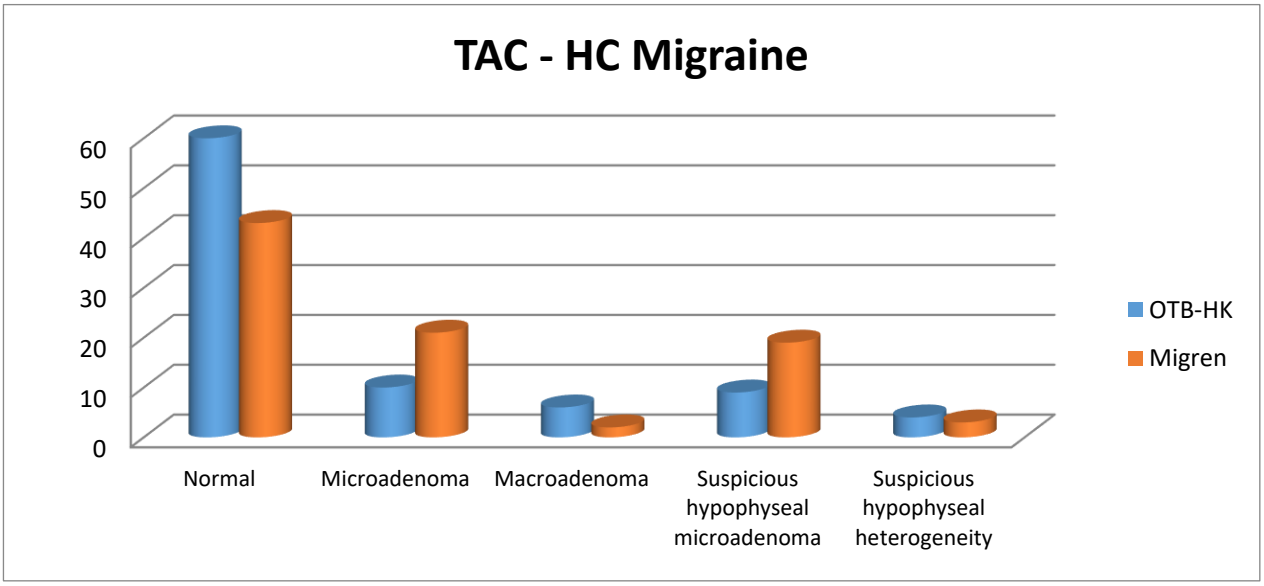

Figure 3. TAC- HC migraine

Discussion

TAC is a group of primary headache syndromes characterized by unilateral headaches and associated with ipsilateral cranial autonomic symptoms (Olesen, 2004). The most frequently observed type of TOC is cluster headache, while paroxysmal hemicrania and SUNCT are less frequently observed. Thee syndromes differ both in terms of the treatment response and the duration and frequency of the attack (Shuu- Jiun et al., 2009).

Hemicrania continua is among the very rarely observed primary headaches accompanied by autonomic symptoms (Olesen, 2004). While the male/female ratio in HC was previously reported as 1/5, it has recently been reported as 1/2-3 (Matharu, 2007). In our patient group, this ratio was ½, which was in line with the literature.

Among the patients with cluster headaches, 80-90% are episodic cluster headaches (ECH) while 10 -20% are chronic cluster headaches (CCH). In our study, 62. 5% were ECH, which indicated a higher frequency than CCH. The male/female ratio in cluster headaches was 2/1(Ozturk, 2011). In our patient group, all of the 8 cluster headache patients were male.

Paroxismal hemicrania is a type of TAC characterized by short attacks. While 80% of the cases are chronic paroxysmal hemicranias, 20% have the episodic form (Levy et al. ,2005). In line with the literature, also in our study, 84. 6% of the patients with paroxysmal hemicranias had CCH, while 15. 4% had ECH. Paroxysmal hemicrania is considered to be prevalent among females (Chinar et al., 2018). Also, in our patient group, it was more frequent among female patients, which was in line with the literature.

In another study by Wang et al. conducted in patients with pituitary adenomas, the association of headaches with the cranial autonomic symptoms were investigated and the frequency of macroadenoma and acromegaly was found to be higher in patients with cranial autonomic symptoms in comparison to those without cranial autonomic symptoms (Jacson et al,. 2009). These findings point out that a structural factor of pituitary adenomas may constitute a probable pathogenesis for headaches in patients with cranial autonomic symptoms. The diagnoses of the patients with autonomic symptoms were found as chronic migraine or probable chronic migraine in 56%, hemicrania continua in 17%, and cluster headache in 17% (Elisabetta, 2009). Unlike this study, in our study, we have investigated the association of pituitary adenomas in patients with autonomic symptoms. Similarly, macroadenomas were more frequently observed in patients with autonomic symptoms in our study, although the result did not reach statistical significance. Also, acromegaly was observed in 1 patient in the group with autonomic symptoms.

Explanations regarding the relationship between headache and pituitary tumors include tension in the dura mater and invasion of the allogenic structures resulting from cavernous sinus invasion. Two studies focusing on the association between the tumor size and cavernous sinus invasion did not find any relationship between these factors and showed that solely physical factors cannot adequately

explain headaches (Matharu, 2007). (Wang et al,. 2009).

Tumor size or invasion is not a satisfactory explanation for cephalgia (Ozturk, 2011). In line with the literature, no relationship was observed between headache and the size and invasion of the tumor in our study.

In a study in the literature where the characteristics of pituitary adenomas and headaches were investigated, the most commonly observed types of tumour were non-functional adenoma with 46. 9%, prolactinoma with 18%, and ACTH-producing adenoma with 17% (Abe T, et al. 1998). In another study conducted on patients with symptomatic TOC, 8 out of the 24 patients with chronic headache (33%) had pituitary lesions, where 7 had functional adenomas and 1 had a non-functional adenoma. Among the 3 patients with paroxysmal headaches, 2 (67%) had functional adenoma; 7 (70%) out of the 10 SUNCT patients were associated with pituitary tumours, while 5 had functional and 2 had non-functional adenomas (Lai, 2009). In our study, except for 1 patient in the TOC-HC group h-who had a hormone-producing adenoma, all the adenomas observed in this group and the groups of migraine patients were non-functional.

It is strongly suggested that the onset of the pituitary tumor headache has a hereditary biochemical – neuroendocrine background (Abe et al., 1998). In some patients with acromegaly-associated headache, somatostatin analogues such as octreotide may act as an instant pain-relief. The same condition is observed with dopamine agonists in patients with prolactinoma. However, more severe headaches have also been reported during these treatments (Wang et al, 2009). (Jacson et al., 2009).

In our study, only one patient was hormonally active and had acromegaly. In this patient, octreotide treatment led to an improvement in the headaches. Our study did not demonstrate a statistically significant relationship between headache and neuroendocrine factors.

Limitations

This is an observational, single-institution study, which had a relatively small sample size and was thus subject to various unaccounted confounders inherent in such an analysis.

Conclusion

In this study, we have investigated both the structural and hormonal role of pituitary lesions in the pathophysiology of autonomic symptoms. Our comparison between patients with TOC-HC and those with migraine without aura unaccompanied by autonomic symptoms did not demonstrate a statistically significant difference in terms of the association with pituitary lesions. These results render both the structural and hormonal effects of pituitary adenomas in the pathophysiology of autonomic symptoms doubtful. Further studies are required for clarification.

Ethics Committee Approval: Ethics committee approval was received for this study from 2012 Clinical Research Ethics Committee of Ankara education Ethics committee.
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Author Contributions: Concept – ŞK, E İ, Design Ö Ç, Supervision Ş K, Materials – B E Ş, Data Collection and/or Processing - ŞK Analysis and/or Interpretation S Ü, Literature Review –Ş K, Writing – ÖÇ, Critical Review – Ş K.
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RESEARCH ARTICLE

The Effect of Calcium Hydroxide and File Sizes on the Accuracy of the Electronic Apex Locator in Simulated Immature Teeth

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Abstract

Objective: The aim of this research was to appreciate the effects of different file sizes and the presence of calcium hydroxide on the correctness of the electronic apex locator (EAL) at simulated immature teeth.

Methods: In this study, 30 maxillary central teeth were used beforehand extracted due to periodontal reasons. The actual root canal length determined with K-File and measured behind 0,5mm from the apex. In order to simulate an immature tooth, the apical of roots were prepared with a diameter of 1,4mm Gates-Glidden drills and K-Files. Working lengths of samples were measured using the Root ZX by 110-140 K-Files. Each measurement was repeated three times and averaged. Then all the samples were filled with calcium hydroxide medicament and working lengths were determined by the same method. Statistical analysis was performed using the Two-way ANOVA.

Results: The measurements were closer to the actual root canal length as the diameter of the file increased at the presence and absence of calcium hydroxide. However, there was a difference between the presence and absence of calcium hydroxide in the determination of root canal length.

Conclusions: In these experimental study conditions, it was concluded that the presence of calcium hydroxide in simulated immature teeth and file size affect the EAL.

Key words: Apex Locater, Root Canal, Calcium Hydroxide

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Introduction

In endodontic preparation and filling of the root canal, apical contraction is considered to be the limiting point of treatment. In epidemiological studies, the working length control is very important and histological studies have reported that optimal improvement is achieved when there is minimal contiguity between the root canal obturation material and the periapical tissue (Sjogren et al., 1990). Accurate determination of working length is a very important component of successful root canal treatment. The traditional methods such as radiography, anatomical averages of canal length, wet paper point and finger sensitivity used to determine root canal length may be insufficient at this point (Sharma and Arora, 2010). Traditionally,

the radiographs taken after the insertion of the file in the root canal are used to determine the working length. Limitations of conventional radiographs include sensitivity technique, magnification, distortion of images, and superposition of anatomic structures. Another disadvantage of radiographs is the danger of ionizing radiation (Gordon and Chandler, 2004). Recently, electronic methods used to determine the root canal length are more widely accepted methods (Baldi et al., 2007).

Cone beam computed tomography (CBCT) is primarily used all the time in the department of oral surgery. For endodontic usage, the definite location and dimension of the pulp cavity can be visualized in 3D for an access-cavity preparation and working length may also be determined in CBCT images and combined with EAL can be useful for working length determination (Jeger et al., 2012).

The anatomical features of the extended apical foramina, development of incomplete root formation, root resorption or non-development of the root can cause difficulties on working length determination (Nguyen et al., 1996). At the same time, different sizes of files, the type of irrigation solution used are among the factors affecting the conductivity of the EAL. Many researchers recommend Ingle's method, the radiographic prediction of the working length 1–2 mm short of the radiographic apex determined from a pre-operative radiograph or from tactile sense while some investigator advocate instrumented to the radiographic apex during treatment of immature permanent teeth with open apices (Ingle, 1994). Although radiographic method was the main method in determining the length of study in the treatment of open apexed teeth, clinical studies and case reports reported variations in the method (Pratten and McDonald, 1996). The European Society of Endodontology recommends the use of an EAL followed by verification of canal length with an undistorted radiograph during root canal treatment (Lost, 2006).

Due to its antibacterial and biological properties, calcium hydroxide is frequently used clinically (Athanassiadis et al., 2007). It is a energetic alkaline material, which has a pH of there about 12,5. This agent has been attributed to various biological properties such as antimicrobial activity, tissue dissolving ability, inhibition of tooth resorption and induction of hard tissue formation and repair. Currently, calcium hydroxide used in endodontic treatment is considered one of the most effective antimicrobial agents. (Freeman and Crapo, 1982).

Clinically, the initial working length determination with EAL is mostly fixed with a small-sized file. However, whether the accuracy of EAL is affected by the use of small-sized files on open apices roots are not explained (Ebrahim et al., 2006). The null hypotheses were tested: (1) In immature root canals, the accuracy of the EAL may be affected by the file size because a small diameter file is likely to leave a space in the root canal, whereas a larger diameter root canal provides a closer fit with the dentin. (2) Due to the inorganic structure of calcium hydroxide, more accurate results can be obtained as it provides more contact between file and the root dentin wall. The aims of this in vitro study were to evaluate the accuracy of Root ZX (Morita, Tokyo, Japan) apex locator measurements with 110 to 140mm diameter files in enlarged simulated immature root canals and to analyze effects of the calcium hydroxide.

Methods

Thirty extracted human maxillary anterior teeth with a single root canal were selected. In this study, previously extracted teeth were used because of periodontal problems. All teeth were examined under stereomicroscope to determine if there was any caries lesion, external resorption, crack or fracture lines. Teeth with caries lesions and calcified canal, root resorption, fractures or cracks were excluded from the study. To ensure standardization of the teeth included in the study, similar teeth were selected and digital calipers were used for this purpose. The apical 5 mm of each tooth was removed using a low-speed diamond bur and standardized to be 16mm long. For all samples, coronal access was prepared using diamond bur in a high-speed handpiece, and the canal patency was checked by inserting #10 K-file (Dentsply Mallefer, Swtzerland).

The specimens were instrumented to ISO size #50 K-File. To simulate immature teeth, the canals were instrumented with Gates Glidden (Shenzhen Denco Medical Co., Ltd.) (no 1–6) until a no.5 Gates Glidden (1,3 mm) could be passed 1 mm beyond the apex and carried out instrumented to ISO size #140 K-File with full working length for providing to 1.4mm apical diameter.

The root canals were irrigated with 5,25 % NaOCl (Microvem, Istanbul, Turkey) during instrumentation with an irrigation needle 2 mm behind the apex. Cylindrical methacrylate molds were used for electronic measurement of working length. Sufficient alginate was placed in the molds and the tooth was embedded in the alginate during

hardening. The tooth was held in this position until the alginate completely cured. During the electronic measurement, the labial clip of the positioner which was stabilized with a transparent adhesive tape was placed in the alginate. All measurements were performed at 30-minute intervals during which time the alginate was kept sufficiently moist. Working lengths of samples were measured using the Root ZX by 110, 120, 130 and 140 K-Files. All measurements were performed by one single operator. Depending on the canal size, a K-file was attached to the file holder and slowly inserted into the root canal until the EAL's digital display read 0,5 mm. The silicone stop on the file was placed in a straight line on the incisal edge of the tooth to be the reference point for the measurement. The file was removed and measured with an endodontic ruler. Each measurement was repeated three times and averaged.

Calcium hydroxide paste (Kalsin, Aktu, Turkey) was mixed homogeneously with a spatula and drawn into the 10cc injector. Each sample was filled completely with calcium hydroxide paste using a 21 gauge needle before measuring. Then all the samples canal lengths were determined by the same method three times and averaged.

Statistical analysis was performed using SPSS 22.0 (SPSS, Chicago, IL, USA) for Windows. A two-way ANOVA was made to examine the effect of different size of file and the presence or absence of calcium hydroxide in the canal on the accuracy of the EAL. The data is the mean ± standard deviation unless otherwise stated. The differences were evaluated by using Shapiro-Wilk's normality test for each design cell of normality and the homogeneity of variance was evaluated by Levene Test. All double comparisons were performed for each simple parent domain with a 95% confidence interval reported and a P value set with Bonferroni adjusted for each simple parent domain.

Results

Apical diameter of the root canal file increases, values near the actual length of the root canal were found and measurements in the presence of calcium hydroxide showed deviations from the actual length of the root canal. In both the presence of calcium hydroxide and measurements without calcium hydroxide, results were obtained closer to the actual canal length as the diameter of the root canal file increases. There was a statistically significant difference in the presence and absence of calcium hydroxide in the root canal with the same diameter K-file ($p<0.05$) (Table 1). The statistical difference was found between 110,130 and 140 K-Files in the measurements performed on the empty root canal. There was a statistically significant difference between 110 and 140 K-Files in the measurements of calcium hydroxide filled root canals ($p<0.05$) (Table 2).

Table 1: Pairwise comparison measurement of the samples with or without calcium hydroxide by the same file.

	Mean Difference			Std. Error	Sig.	P
110mm	Canal Filled with Calcium Hydroxide	Blank Canal	0,513	0,123	0,001	<0,05
120mm	Canal Filled with Calcium Hydroxide	Blank Canal	0,542	0,127	0,000	<0,05
130mm	Canal Filled with Calcium Hydroxide	Blank Canal	0,634	0,113	0,000	<0,05
140mm	Canal Filled with Calcium Hydroxide	Blank Canal	0,507	0,108	0,000	<0,05

Table 2: Pairwise comparison of different root canal files with the same samples.

			Mean Difference	Std. Error	Sig.
Empty Canal	110mm	120mm	0,191	0,104	0,503
		130mm	0,345	0,099	0,015*
		140mm	0,374	0,099	0,008*
	120mm	130mm	0,154	0,047	0,024*
		140mm	0,183	0,074	0,146
	130mm	140mm	0,029	0,052	1
Canal Filled with Calcium Hydroxide	110mm	120mm	0,162	0,104	0,828
		130mm	0,224	0,099	0,213
		140mm	0,38	0,099	0,007*
	120mm	130mm	0,062	0,047	1
		140mm	0,218	0,074	0,054
	130mm	140mm	0,156	0,052	0,045*

*Statistically significant *(P<0.05)* compared to different file sizes

Discussion

Determining the correct working length of immature teeth during root canal treatment is necessary to promote complete cleaning and disinfection of root canals. For this purpose, EALs are able to overcome some limitations of images obtained by radiographic methods.

With developing technology, EALs are an essential part of endodontic treatment in clinic usage. The best results were reached with the latest generation devices, such as Root ZX (Dunlap et al., 1998). Electronic measurements of working length may be influenced by different factors. When the apical constriction is destroyed as a result of apical root resorption, development of incomplete root formation it is very difficult to determine the working length by radiographic methods alone (Shabahang et al., 1996).

Since there may be variability in the measurement of immature teeth using different types of EAL, one type of EAL was used in our study to standardize the measurements.

In their study, Hulsmann and Pieper showed that EAL measurements in roots with large apical foramens were significantly shorter than the actual canal length (Hulsmann and Pieper, 1989). Even if the anatomic apical constriction is impaired, the Root ZX constantly shows that the file tip is protruding from the apical. This narrowing confirms previous evidence that it defines the narrowest diameter of the canal, regardless of anatomical or mechanical occurrence (Angwaravong and Panitvisai, 2009). Electronic measurements of working length may be influenced by different file size. According to the previous studies, more successful results were obtained in electronic measurement methods as the diameter of the file was increased (Ebrahim et al., 2006; Herrera et al., 2011). In our study, more accurate results were obtained with the larger diameter files trapped in the narrowest diameter of the root canal which supports previous studies.

Many irrigation solutions are used in endodontic treatment because of its properties such as antibacterial, lubricant and ability to dissolve vital tissue (Ebrahim et al., 2006). However, the presence of any fluids may effected EAL's measurements. Some researchers have shown that the correct measurement of EAL is independent of the concentration of NaOCl solution and, thanks to its high electrical conductivity and its ability to penetrate dentin tubules, the root canal walls reduce the electrical impedance and also have the potential to create better electrical contact with periapical tissues (Jenkins et al., 2001; Tinaz et al., 2002; Pilot and Pitts, 1997).

Removal of all bacteria from the root canal system is normally completed by mechanical instrumentation that sutained with various irrigating solutions and antibacterial agents. Calcium hydroxide is highly advised among these intracanal medicaments and widely accepted as an interappointment antibacterial agent, that proves a pronounced antibacterial activity against most of the bacterial species defined in root canal infections (Bystrom et al., 1985).

The difference of measurement of root canal obtained by particular researchers may, according to

Kolanu, not be caused by the size of the foramen itself, but also by the surface of contact of the active electrode with the walls (Kolanu et al., 2014).

Due to the inorganic nature of calcium hydroxide, the same samples were filled with calcium hydroxide by the hypothesis that more contact between the file and the root dentin wall will be achieved and more accurate results can be obtained. In the samples filled with calcium hydroxide, as the diameter of the file increased, the contact between the dentin wall and the file increased, the actual size of the canal was found closer to the values (Table 2). However, there was a statistically significant difference in two different measurements with 140 mm files. In the same measure, the calcium hydroxide group was found to be shorter than the actual length of the root canal (Table 1). Due to the irregular nature of the canal and the non-uniform distribution of the calcium hydroxide paste, incorrect results may have been obtained due to the incomplete contact between the dentin wall and the root canal file. In a study, it has been reported that calcium hydroxide residues have a negative effect on electronic measurement methods (Uzunoglu et al., 2015).

Residuel calcium hydroxide paste that obtained after irrigation may also effect the accuracy of EAL. This measurement did not reported. This could be the limitation of the present study

The removal of calcium hydroxide from radicular dentin is essential in order to provide the unity of the root canal seal. On the other hand, none of the irrigation methods used in a previous study has been reported to remove calcium hydroxide from the canal wall, leaving 25 to 45% of the surface of the walls covered with the calcium hydroxide dressing (Lambrianidis et al., 1999). More extensive studies are needed to assess the effect of different irrigation solutions and methods used to remove calcium hydroxide from the root canal wall on the accuracy of the EAL.

Conclusion

When compared to direct canal measurement, the Root ZX apex locator with larger apical diameters files tends to convenient working length of simulated immature teeth.

In the presence of calcium hydroxide in the samples, the results were found to be shorter than the actual working length.

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CASE REPORT

Isolated Maxillary Sinus Aspergilloma Associated with Tooth Extraction: Case Report
Short title: Fungus Ball of Maxillary Sinus

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Abstract
Aspergillosis is a fungal infection mainly affects the nasal cavity and paranasal sinuses in maxillofacial region. Aspergillus infection can be seen in three clinical forms as non-invasive, invasive and allergic. The non-invasive form of aspergillus also called as aspergillus mycetoma, aspergilloma or fungus ball, commonly seen in healthy individuals. Maxillary sinus aspergilloma may occur after treatments of antral teeth. Although usually asymptomatic maxillary sinus aspergilloma in some instance may exhibit clinical symptoms such as swelling, purulent discharge, chronic sinus pain, nasal congestion, headache which are similar to the other sinus infections in symptomatic patients. This case report presents the diagnosis and surgical management of maxillary sinus aspergillus infection that occurs after tooth extraction.
Key words: Fungus, paranasal sinus, aspergillosis

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Introduction

Aspergillus is a fungus commonly found in nature and also the most common fungal pathogen isolated from the paranasal sinuses (Khongkhunthian and Reichart, 2001; Harada et al., 2017). Aspergillus infection can have three clinical forms as non-invasive, invasive and allergic (Sato et al. 2010). The non-invasive form of aspergillus, mostly seen in healthy individuals, is called as aspergillus mycetoma, aspergilloma or fungus ball (Khongkhunthian and Reichart, 2001). Aspergilloma can affect all paranasal sinuses, but it is especially seen in maxillary sinus (Shin et al., 2016). Unlike other paranasal sinuses predisposing local factors that contribute the occurrence of this fungal infection in the maxillary sinus are considered as dental procedures that cause perforation of the sinus membrane such as tooth extraction and endodontic treatment

(Khongkhunthian and Reichart, 2001; Martins and Ribeiro Rosa, 2004).

The clinical course of maxillary sinus aspergilloma is usually asymptomatic (Harada et al., 2017). However, in symptomatic cases, the clinical symptoms of aspergilloma are usually not specific and include nasal discharge, chronic sinus pain, nasal congestion, headache, and orbicular pain (Sohn et al., 2009; Guivarc'h et al., 2015). Radiographically, aspergilloma shows a dense, opaque, foreign body appearance in the maxillary sinus and is usually detected during routine dental examination (Torul et al., 2018). Providing the natural sinus drainage after removal of the lesion by Caldwell-Luc operation or endoscopic surgical techniques is sufficient for the treatment of aspergilloma. Generally, anti-fungal therapy is not required (Khongkhunthian and Reichart, 2001; Harada et al., 2017).

In this case report, the diagnosis and surgical treatment of maxillary sinus aspergilloma that occur after tooth extraction, is presented.

Case Report

A 40-year-old female patient presented to our clinic with temporomandibular joint problems. The patient's general health was unremarkable. In clinical examination, no pathology was observed. However, panoramic radiographic examination showed a radiopaque foreign body located in the right maxillary sinus (Figure 1). Cone Beam Computed tomography (CBCT) was obtained for further investigation and the exact location of the hyperdense foreign body in the right maxillary sinus was detected. No evidence of bone destruction was seen on the sinus walls on CBCT (Figure 2). Because of the history of occasional pain from the right maxillary sinus region excision of the foreign body under local anesthesia was planned.

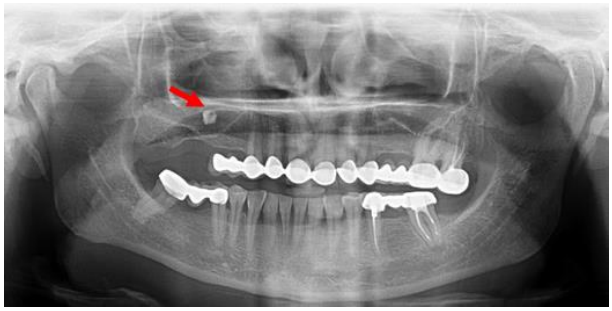

Figure 1. Panoramic radiograph showing the radiopaque mass in the right maxillary sinus.

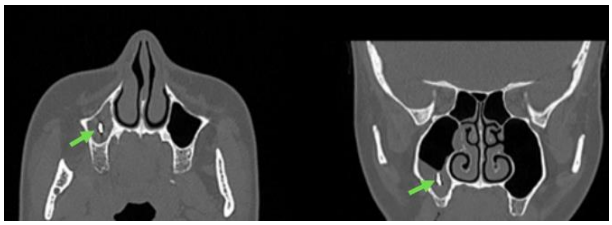

Figure 2. Axial and coronal CBCT images shows iron-like opacity.

After informed consent was obtained, local anesthesia was administered to the region and the full thickness flap was raised. The foreign body was removed with the Caldwell-Luc procedure (Figure 3). After the removal of foreign the body 9 ml blood harvested from the patient. Concentrated Growth Factor (CGF) was obtained by placing the blood into the centrifuge. CGF was placed into the sinus and the bony window in the lateral wall. Primary closure was performed subsequently (Figure 4). After operation antibiotic, analgesic, mouthwash and decongestant drugs were prescribed to the patient. Excised specimen was sent to histopathological examination (Figure 5). The histopathology report revealed the diagnosis as Aspergillus (Figure 6). Six months after surgery, she had no clinical and radiographic evidence of infection (Figure 7).

Figure 3. Removal of the foreign body.

Figure 4. Placement of CGF.

Figure 5. Excised specimen.

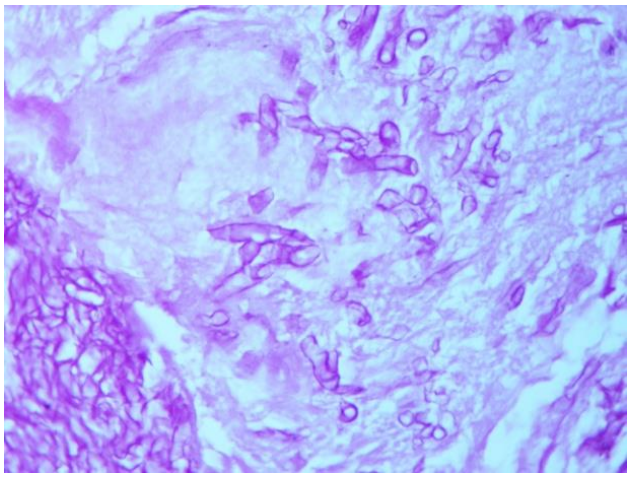

Figure 6. Histopathological finding shows fungus hyphae (PASX1000).

Figure 7. Postoperative panoramic radiograph at 6th months.

Discussion

Aspergillosis mainly affects the nasal cavity and paranasal sinuses in maxillofacial region (Urs et al., 2015). It is accepted that aspergillus infection in these regions occurs as a result of inoculation of aspergillus spores to paranasal sinuses by inhalation, and become pathogenic in hypoxic cases where sinus drainage is deteriorated (Sohn et al., 2009; Shin et al., 2016). Some researchers have suggested that the higher incidence of aspergilloma in the maxillary sinus is caused by the dental factors in addition to other etiological factors that contribute to the occurrence of the infection. According to these authors, the perforation of the maxillary sinus membrane with the dental procedures leads Aspergillus spores which are lack of lytic enzymes, to reach the sinus and cause the infection here (Guivarc'h et al., 2015; Urs et al., 2015; Harada et al., 2017). Different cases of aspergilloma associated with dental procedures such as dental implants, tooth extraction, root canal treatment and alveolar grafting have published in the literature previously (Sohn et al., 2009; Sato et al., 2010; Urs et al., 2015; Harada et al., 2017;). Harada et al. (Harada et al., 2017) reported a case of aspergilloma associated with the migrated dental implant. In another case aspergilloma associated with dental material was reported by Giardino et al. (Giardino et al., 2006). In our case, we think that Aspergillus inoculate to the sinus as a result of the perforation of the sinus membrane by tooth extraction. Also, the root canal filling material which is pushed into the sinus during extraction has provided available conditions to Aspergillus spores can be pathogenic.

Aspergillus, which is usually present in the sinus without symptoms, has been reported to cause chronic pain, swelling and nasal obstruction in some cases (Sohn et al., 2009). Urs et al. (Urs et al., 2015) reported swelling and pain in nasomaxillary area after endodontic treatment. In another case Martins

and Ribeiro Rosa (Martins and Ribeiro Rosa, 2004) reported recurrent swelling and purulent discharge after root canal treatment of right upper maxillary molar. In our case, chronic pain was present in the right facial region.

Plain radiographs are used in the diagnosis of fungal infection of the sinuses. A radiopaque appearance similar to that of metal in the maxillary sinus is diagnostic in panoramic radiography (Falworth and Herold, 1996). However, CBCT can also be used to assess the exact size of the fungus ball, bone involvement and erosion (Harada et al., 2017; Torul et al., 2018). In addition, magnetic resonance imaging can show the changes in soft tissues and help the differential diagnosis of aspergilloma (Falworth and Herold, 1996; Harada et al., 2017). In our case, in addition to panoramic radiography, CBCT examination was performed to determine the exact location of aspergilloma.

Caldwell-Luc procedure and endoscopic sinus surgery techniques are used in the treatment of aspergilloma. The removal of the foreign body from the area and re-orientation of natural sinus drainage is sufficient in the treatment of aspergilloma (Khongkhunthian and Reichart, 2001; Burnham and Bridle, 2009). After successful surgery, anti-fungal therapy is usually not necessary. If the opacities in the maxillary sinus persist or the patient is symptomatic for a long time after surgical treatment, an adjuvant antimycotic drug seems to be required (Sohn et al., 2009; Sato et al., 2010; Harada et al., 2017). In this case, the Caldwell-Luc procedure was preferred to remove aspergilloma. CGF was applied to the sinus to support and accelerate postoperative recovery. An uneventful period was observed after the operation.

Conclusion

Maxillary sinus aspergilloma is usually asymptomatic and can be overlooked. Also, in symptomatic cases, the symptoms may be confused with other infectious conditions affecting the maxillary sinus and unnecessary treatments may be performed as a result of underestimation of the fungal etiology. Therefore, clinicians should follow up patients after dental procedures affecting the maxillary sinus to prevent such conditions; and should be considered the fungal etiology in the case of a maxillary sinus infection.

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REVIEW

Postoperative Nausea and Vomiting: Risks, Prophylaxis, Non-Drug Alternative Methods

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Abstract

Nausea and vomiting are among the most common complaints in the postoperative period. The type of surgical technique and the site of intervention, as well as the anesthetic drugs and methods directly contribute to the rates of postoperative nausea and vomiting (PONV). One of the most important factors affecting the frequency of postoperative nausea and vomiting is the anesthetic drugs and methods used. Many antiemetic drugs are used to prevent postoperative nausea and vomiting. With the use of these drugs, side effects especially such as delayed recovery from anesthesia, dry mouth, undesirable changes in blood pressure and the occurrence of extrapyramidal symptoms are seen. Several antiemetic drugs are commonly used for the treatment of postoperative nausea and vomiting; including scopolamine, chlorpromazine, diphenhydramine, metoclopramide, promethazine, and ondansetron. Low-dose haloperidol and metoclopramide are effective in opioid-induced nausea and vomiting. Dexamethasone reduces the incidence of postoperative nausea in the first 6 hours after surgery. Oral intake starts in patients with severe vomiting parenteral fluid and electrolyte treatment is required. Sometimes provide may need gastric decompression with nasogastric tube. Parenteral fluid and electrolyte treatment and in severe cases gastric decompression with a nasogastric tube may be necessary. Alternative medicine such as acupuncture and acupressure can help prevent or reduce postoperative nausea and vomiting. The aim of this review is to examine the medical therapies and drug-free methods used for the treatment of PONV in the light of the literatüre

Key words: postoperative nausea, vomiting, drug, acupuncture

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Introduction:

Postoperative nausea and vomiting (PONV) are the most frequent early complications of general anesthesia, causing dissatisfaction in the anesthesiologist and the patient. A considerable number of patients describe PONV among the most discomforting postoperative complaints, even more unpleasant than pain (Macario et al.,1999; Macario, Weinger, Truong et al.1999; Gan et al.2003). In the literature, the incidence of PONV is reported to be 25-30% in the absence of pharmacological treatments and 70% in high-risk patients in the general population. Despite these high rates, completely effective prevention and treatment strategies have not been established for PONV, yet

(Huang et al.,2001; Gan et al.,2003). However, several risk factors and preoperative prophylactic antiemetic treatment strategies have been defined in recent studies and published guidelines (Apfel et al.,1999; Gan et al.,2003; McCracken et al.,2008). The reported risk factors of PONV include the type of surgery, female gender, being a non-smoker, previous experiences of PONV or motion sickness, and postoperative opioid use (Apfel et al.,1997; Myklejord et al.,2012). In the guidelines published in 2003 and 2008; symptomatic treatment with antiemetics is recommended for low-risk patients, prophylactic antiemetic use is recommended only for the treatment of moderate-to-high-risk patients, and drug combinations are recommended only for the high-risk patients (Gan et al.,2003; McCracken et al.,2008). For preventing the development of PONV, different classes of antiemetics are used; including dexamethasone, 5-hydroxytryptamine, serotonin (5-HT3) receptor antagonists, and dopamine receptor antagonists. These pharmacological agents have their mechanisms of action independent from each other. It is reported that the efficacy of antiemetics is limited in monotherapy but enhanced in combined use (Henzi et al., 2000; Apfel et al., 2004). Particularly, the combined use of dexamethasone with 5-HT3 receptor antagonists is known to be associated with an enhanced antiemetic activity (Eberhart et al., 2000; Henzi I, Walder B et al., 2000).

The aim of this article is to review the pharmacological and drug-free methods used for the treatment of PONV in the light of the literature.

1. Definition of Postoperative Nausea and Vomiting and General Information:

Complaints of nausea and vomiting developing in the first 24 hours after surgical intervention are defined as postoperative nausea and vomiting (PONV). PONV is a common condition associated with the administration of anesthesia, leading to patient dissatisfaction. It may sometimes become severe enough to contribute to intolerably high heart rates or blood pressure, increased intrathoracic pressure, and central venous pressure. PONV is reported at rates as high as 80% in high-risk patients and at a 30% rate in the general population (Grabowska-Gaweł et al.,2006). The most common patient-related risk factors include being a middle-aged woman, a non-smoking woman, and having a history of PONV or motion sickness. The treatment-associated factors to develop PONV include the use of volatile anesthetics (inhalation anesthetic agents), prolonged surgery, N_2O use, and administration of postoperative opioids. The type and the site of surgery are among the major players, too; with ocular, tympanic, intracranial, abdominal, and gynecologic operations posing a high risk to develop PONV (Grabowska-Gaweł et al.,2006). Grabowska-Gaweł et al. study on patients receiving general anesthesia reported that nausea and vomiting were observed in 22.7% and 13.2% of the patients respectively and that 14.2% of the patients had developed both symptoms (Grabowska-Gaweł et al.,2006).

1.1. Pathophysiology

The pathophysiology of nausea and vomiting involves complex mechanisms and has not been completely understood, yet. Nausea is a conscious sensation and vomiting is a complex reflex. The former requires the involvement of cortical structures and the latter is controlled by the medulla oblongata. The act of vomiting requires coordination of systems including the involvement of emesis-related afferents combined with the actions of the respiratory, gastrointestinal, and abdominal muscles. This whole process is mediated by a specific area in the central nervous system, namely the vomiting center. The current hypothesis suggests that there are neurons in the medulla oblongata, specifically organized for being activated by the nausea center; which coordinates nausea and emesis to occur sequentially. This hypothesis favors the concept of a "central stimulus pattern", suggesting that it is more acceptable than the "vomiting center" concept (Aygin et al.,2004). Even though the nucleus tractus solitarius (NTS) and other specific nuclei of the reticular formation (including the respiratory nuclei) are considered to be the important sites for generating emesis, the central stimulus pattern is not well defined (Aygin et al.,2004). It is reported that NTS activates the central stimulus pattern and surrounding neuron groups to generate the autonomic and motor responses leading to vomiting (Çekmen et al.,2003).

The following anatomical structures are mainly involved in projectile vomiting;

1. The vagal afferent fibers of the gastrointestinal tract
2. The vestibular system
3. The cerebral cortex, thalamus, and hypothalamus
4. The area postrema (AP)

Enteroendocrine cells induce serotonin synthesis when circulating drugs or toxins are present in the system. The synthesis of serotonin stimulates the vagal afferent fibers of the gastrointestinal tract. Other types of emesis, psychogenic vomiting, and vomiting induced by smelling or visual stimuli are generated by the stimulation of thalamus, hypothalamus, the respective areas in the cortex, and the vestibular nuclei; which receive motion-dependent sensory data (Aygin et al.,2004).

Recent research has focused on AP and the chemoreceptor trigger zone (CTZ). AP is a specific area located at the base of the fourth ventricle in the medulla oblongata. AP is rich in blood vessels, but it does not have a specific blood-brain barrier. These properties render the chemoreceptors in the AP highly sensitive to the effects of circulating emetogenic agents in the blood and cerebrospinal fluid. Therefore, AP plays an important role in drug-induced vomiting. AP receives stimuli from the emetic receptors in a variety of ways; including the transmission of stimuli via the direct afferents, and the visceral vagus nerve. Molecules in the blood circulation and autonomic stimuli transmitted by the descending pathways from the hypothalamus stimulate AP, too (Aygin et al.,2004). Consequently, the vagus nerve and the neuron groups associated with nausea and vomiting are activated by the afferent fibers and interaction of various neurotransmitters; which are serotonin (5-HT), dopamine (D), histamine (H), Substance P, acetylcholine, and opioids. The receptors of these neurotransmitters are available in the vagal afferents (5-HT3 receptors), vestibular nucleus (M3/M5 muscarinic acetylcholine receptors and H1 receptors), AP (μ, 5-HT3-, and D2-opioid receptors), and NTS (μ receptors, 5-HT3, neurokinin-1, and Substance P receptors) (Aygin et al.,2004).

Disadvantages of PONV include the discomfort in the patient, prolonged length of hospital stay, and delays in recovery (Çekmen et al.,2003). Besides these untoward consequences, severe PONV is associated with consequent fluid and electrolyte loss, aspiration into airways, contamination of the surgical site, increased strain in the sutured incision site, and increased intraocular and intraabdominal pressure (Kayhan Z ,2004; Wesmiller et al.,2017).

The antagonization of neuromuscular blockade, which was previously thought to be a risk factor for PONV, showed that obesity and anxiety were not associated with PONV (Gan et al.,2014). Postoperative pain, hypotension, capacity for movement and fluid intake may also predispose to PONV (Ozatamer et al.,2010). Simplified risk models have been developed in order to calculate the PONV risks of adult patients (The Apfel score, Table 1). In the absence of any risk factors, the PONV risk is assumed to be 10%. While the female gender, non-smoking, history of PONV/motion sickness, and postoperative opioid use is associated with a 20% risk for PONV, it is accepted that the risk increases by 20% with the addition of each risk factor and reaches 80% in the presence of the four factors (Gan et al.,2014) (Table 1).

Table 1. Scoring system to predict postoperative nausea and vomiting in adults (The Apfel Scoring System)

Risk factor	Scoring
Female gender	**1**
Non-smoking	**1**
History of PONV/Motion sickness	**1**
Postoperative opioid use	**1**

The total Apfel scores may range from 0 to 4 (Gan et al.,2014).

Risk factors for PONV can be categorized under three main titles as follows:

1. Patient-dependent risk factors: Female gender, history of previous PONV, history of smoking, history of motion sickness, and age.
2. Anesthesia-dependent risk factors: Receiving inhalation anesthetics, duration of anesthesia, nitric oxide use, and postoperative opioid use
3. Surgery-dependent factors: Cholecystectomy, laparoscopic surgical procedures, and gynecological surgery (Diemunsch et al.,2007).

1.2. Evaluation of Postoperative Nausea and Vomiting

It is extremely difficult to evaluate PONV objectively since these complaints are subjective experiences of the patients. However, the Abramowitz Emesis Scoring system may help grade the severity of vomiting (Abramowitz et al.,1983) (Table 2).

Table 2. Abramowitz Emesis Scoring System

Evaluation	Score
No vomiting	**0**
Slight vomiting (1 times)	**1**
Moderate vomiting (2 times)	**2**
Severe vomiting (4 times)	**3**
Persistent vomiting	**4**

1.3. Treatment

The treatment and prevention of PONV are the major components of the management of these complaints. Even though some anesthetists prefer to administer routine prophylaxis to the surgical patients to prevent PONV; this strategy brings along additional costs as well as the risks of administrating extra drugs. A prophylactic strategy can be the administration of a balanced crystalloid solution as dehydration in the perioperative period is an important risk factor to develop PONV. However, it is more critical to perform an individual risk evaluation in each patient instead of administering routine prophylactic management strategies (Table 3) (Apfel et al.,2012).

Table 3. Risk evaluation with the Apfel scoring in terms of PONV incidence

Score	PONV Incidence
0	10
1	21
2	39
3	61
4	79

Most patients receiving prophylactic drug treatment to prevent PONV do not benefit and 1–5% of these patients may experience mild side effects such as headache, sedation or dry mouth (Carlisle et al.,2006). If vomiting occurs despite the prophylactic management strategies, it will mean that the pharmacological agents used for prophylaxis will not be effective for the treatment (Kovac et al.,1999).

Kappen et al. conducted a single center randomized study on 12,032 patients and demonstrated the efficacy of the risk scoring system in identifying the patients to administer prophylactic treatment for PONV (Kappen et al.,2014). Antiemetic prophylaxis is recommended for moderate and severe risk patients to develop PONV. If two drugs are to be used in combination for the prophylaxis, they should be selected from different pharmacological classes. Combination treatments are usually reported to be more effective than monotherapy (De Oliveira et al.,2011).

Although the type of surgery is an important risk factor in the development of postoperative nausea and vomiting, it has not yet been proven to be an independent risk factor. The incidence is higher in some types of surgery (e.g. abdominal surgeries) due to prolonged exposure to general anesthesia and high-dose opioid use. Recent studies have shown that laparoscopic, gynecologic and cholecystectomy operations in terms of PONV are high-risk surgeries In summary, surgical-related factors and anesthesia-related factors can create postoperative nausea and vomiting with a combined effect (Apfel et al.,2012) The drugs used to treat PONV are briefly mentioned in the following section.

1.4 Drugs Used in PONV Treatment

1.4.1 5-Hydroxytryptamine (5-HT3) antagonists

Ondansetron, granisetron, dolasetron are the 5-HT3 agonists and they are used in daily doses of 4-8 mg. These pharmacological agents act completely in CTZ, inhibiting the 5-HT3 receptors. Common side effects of these medications include headaches, flushing, constipation, elevations in the levels of liver enzymes, and bradycardia. These medications are recommended to be given to patients at the end of surgery (De Oliveira et al.,2012). Ondansetron is reported as the gold standard treatment option in many studies in the literature (Jokela et al.,2009).

1.4.2 Dexamethasone

Dexamethasone is an effective antiemetic agent at daily doses of 4-8 mg. It is recommended to administer dexamethasone immediately after the induction of anesthesia to obtain the maximum effect. The administration of rapid bolus doses of dexamethasone to awakened patients may cause irritability, perineal pain, and burning sensation. Other common postoperative side effects of dexamethasone include euphoria and insomnia (De Oliveira et al.,2012).

1.4.3 Cyclizine

Cyclizine is the only pharmacological agent with H1 antagonist activity displaying antiemetic properties. Cyclizine is usually recommended to be administered at a daily dose of 50 mg. Cyclizine has

been in use for a long time with a well-established safety and efficacy profile; however, it is not devoid of some serious adverse effects limiting its use. These adverse events include flushing and tachycardia, injection pain, and phlebitis (Habib et al.,2004).

1.4.5 Phenothiazines

Prochlorperazine and promethazine are the pharmacological agents in this class. Their use for the treatment of PONV is limited due to their side effect profile due to the antidopaminergic and anticholinergic side effects. Short-term administration of these medications is most commonly associated with extrapyramidal symptoms (EPS), acute dystonia, and sedation (anti-dopaminergic effects). The use of these medications should be avoided in patients with Parkinson's disease. Anticholinergic effects manifest with dry mouth and tachycardia. The effective dose of prochlorperazine is reported to be 12.5 mg administered by an intramuscular injection for salvage treatment or prophylactic treatment of PONV (Schaub et al.,2012).

1.4.6 Droperidol

Droperidol antagonizes the dopamine D2 receptors in CTZ. Droperidol is available as a 2.5 mg/ml solution. The recommended dose for the antiemetic effect is 0.625-1.25 mg to be administered at the end of the surgical intervention. However, cardiac complications are reported even at low doses. Other adverse events include anxiety and EPS, which are dose-dependent, and which may occur 12 hours after the administration (Lamond et al.,1998).

1.4.7 Metoclopramide

Metoclopramide is a weak D2 receptor antagonist in CTZ. Although the efficacy of metoclopramide has been reported at doses as high as 20-30 mg, a single 10 mg dose has been shown to be effective after intravenous administration for the prophylactic treatment of PONV in the early period (Murphy et al.,2006). EPS may develop 72 hours after a single dose. Due to the risk of developing EPS, metoclopramide use needs to be avoided in young female patients (Murphy et al.,2006).

1.4.8 Modes of administration of anesthetic agents

Anesthetic agents can play a major role in the development of PONV, demonstrating different effects at different doses and modes of administration. For instance, a bolus administration of propofol for induction has not been associated with the development of PONV; however, total intravenous anesthesia has been shown to reduce the risk of PONV. Therefore, administration of total intravenous anesthesia is recommended in high-risk patients in combination with the use of appropriate antiemetics agents when indicated (Habib et al.,2006).

1.4.9 Miscellaneous

Opioid receptor antagonists, such as naloxone at very low doses (0.25 mg/kg/h) have been demonstrated to reduce the risk of developing opioid-associated PONV after the use of opioids to obtain analgesia (Melzack et al.,1977). There is an increasing interest in neurokinin-1 (NK-1/substance P) antagonists. The representing drug in this class is aprepitant, characterized by a low risk profile, favorable oral availability, and significant efficacy. These properties suggest the use of molecules in this pharmacological class as attractive options when appropriate. Combination of 5-HT3 and NK-1 receptor antagonists in the treatment for up to 48 hours are reported to reduce the PONV rates significantly (Habib et al.,2006).

2. Acupuncture in PONV Treatment

Acupuncture is applied to patients by stimulating the skin and subcutaneous muscle tissue. Acupuncture points are characterized by lower electrical resistance and higher electrical potential compared to the surrounding skin. This principle is used for locating the acupuncture points with the use of detectors. Studies on the bioelectrical principles of acupuncture have demonstrated that it is possible to accurately measure the differences in the levels of resistance and electrical potentials. It has been reported that these findings can be used for diagnostic purposes, too. Furthermore, almost 70-80% of the acupuncture points are associated with the trigger points and most of these points were found out to be identical with the motor points of the muscles (Liao et al.,1975; Bokhari et al.,1977). The P6 (Nei Guan) point is the most commonly used acupuncture point for the PONV treatment (Chernyak et al.,2005), located between the flexor carpi radialis and palmaris longus tendons on the middle finger line at an approximately 6 mm depth and 2 thumb breadths (2 cm) away from the inner curve of the wrist (Chernyak et al.,2005).The P-6 point shown in Figure 1 (Figure 1 taken from

Guyton Physiology major text book;Guyton et al.,2001).

Figure 1. Anatomical Localization of P-6 Point

The efficacy of antiemetics is currently limited for the treatment of PONV. The combination of rare complications along with the relatively low efficacy of the pharmacological agents has increased interest in alternative medicine methods for PONV treatment (Dundee et al.,1986). Acupuncture is among the most commonly used and studied alternative medicine method in anesthesia for the treatment of PONV (Al-Sadi et al.,1997; Kotani et al.,2001; Hickman et al.,2005). Stimulation of the Nei Guan (Pericardium-6) point is effective in alleviating the severity of PONV (Al-Sadi et al.,1997; Wang et al.,2002; Hickman et al.,2005). It is more effective in preventing the development of early postoperative PONV compared to late postoperative PONV (Fassoulaki et al.,1993; Tavlan et al.,1996; Wang et al.,2002). Stimulation of this acupuncture point with electric current by using appropriate acupuncture techniques is reported to reduce the severity of postoperative nausea (Zarate et al;2001). The efficacy of stimulating the Nei Guan point in adults and children has been shown to be same as the ondansetron therapy in the treatment of PONV (Fassoulaki et al;1993; Rusy et al., 2002; White et al., 2002).

A variety of acupuncture stimulation methods have been tried for the treatment of postoperative nausea and vomiting. Some of these include the combination of acupuncture and acupressure, acupressure treatment alone, acupressure with electric stimulation, laser stimulation, and capsicum (Capsaicin) therapy (Tavlan et al.,1996; Shenkman et al.,1999). However, the best stimulation method has not been established, yet. Administration of non-invasive treatment methods like the aforementioned acupuncture stimulation techniques is easier, painless, and better tolerated by the patient; however, the number of studies reporting inefficacy of these methods is considerable and studies in the literature report partial efficacy only on nausea but no efficacy on vomiting. Therefore, non-invasive methods have been suggested to be less effective and inadequate in the literature (Dundee et al., 1986; Tavlan et al., 1996).

Conclusion

PONV remains to be a factor contributing to morbidity and significantly deteriorating patient comfort. To prevent the morbidity, a risk classification should be performed to estimate the development and severity of PONV by evaluating the patient with the use of the reliable scoring systems in the preoperative period. A variety of perioperative and postoperative management strategies (such as the use of prophylactic antiemetic drugs, avoiding the use of opioids for analgesia, etc.) can be employed based on the results obtained by using the risk classification systems. Alternative methods such as acupuncture and acupressure can be administered for the treatment of PONV, too, promising efficacy with no side effects. We believe that it will very beneficial for anesthesiologists to be informed of the acupressure point P-6 (Nei Guan) to offer this highly simple, inexpensive, and side-effect-free alternative management strategy for the treatment and prophylaxis of PONV.

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REVIEW

Screen: Subject of all Information Technology Addiction

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Abstract
The dizzying speed of the change in technology affects the human life in the same movement. Studies which investigate these effects and the increase in that rate become slow and insufficient against the speed of the technology. Although it is not yet considered as an addiction in DSM, it is widely accepted by field researchers that excessive and uncontrolled use of information technologies can cause addiction on individuals. The current study which was conducted by the compilation method includes determining the subtypes of screen addiction as a very up to date and controversial topic, revealing and classifying the underlying reasons of these subtypes and screen addiction in general with the method of compilation. For this purpose, 84 studies which were conducted in Turkey and abroad were examined. As a result of the examination, screen addiction was thought to be divided into subtypes such as internet addiction (gambling, shopping, sexuality, general), media addiction (traditional media, social media), digital game addiction (online, offline, single-player, multiplayer, depending on the type), addiction of technological tools (computers, smartphones, television, tablet, VR and so on.). Consequently, dynamics which are common in these subtypes and cause addiction alone or in combination are digital interaction, virtual environment and screen that transport all these dynamics.
Key words: Technology, screen, addiction, effect

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Introduction

In 21. Century's "Information Age", human being is in an intense and personal interaction with technology in general and information technology specifically unlike the "Agriculture" and "Industry" ages (Yalçın, 2003; Tonta & Küçük, 2005). It is an undeniable fact that information technologies as computer, television, telephone, tablet entered into many aspects of human life such as transportation, communication, education, health; affected, changed and made human life easier in biological, psychological and social aspects. However, research investigating the implications of digital technology in the lives of individuals is inadequate to capture the speed of technological change. Previous research on radio and television gave way to studies on computer and then after smartphones

(Pagani et al., 2010; Sussman & Moran, 2013; Domoff et al., 2017; Cha & Seo, 2018). Today, due to the development in information technology it is possible to have high-level interaction connecting to the internet, create, share or engage in a virtual environment, consume high-level imagery stimulus, and there are many different technological tools from a fridge as the simplest of kitchen appliances to the transportation or virtual reality or augmented reality glasses and so they are still developed. The main factor in the use and presentation of all these tools described is the "screen". Mobile technological tools, in which individuals are getting more intertwined, affect every moment of individuals as in the case of phubbing (Karadağ et al., 2016).

Screen refers to the technology that shows all kinds of virtual images being in interaction. Today, screen sometimes offers a unidirectional interaction and sometimes a bi-directional interaction between individual's own reality and virtual facilities that are offered by technology such as televisions, computers, phones, tablets, projectors, virtual reality and augmented reality. Black and white single-channel television, which was entered into human life, is now across the individual at home, in the pocket at the outside, on the table at work, so it is always everywhere in life. Screen has become an indispensable part of the life and used excessively and uncontrolled with negligent and allowing attitudes of families especially at younger ages (Brindova et al., 2014). At this point, attitudes of parents on using screen affect children at a damaging rate (Rideout & Hamel, 2006). Former minister of health Tim Loughton estimated that a child would spend a whole year with screen until 7 years old in United Kingdom (Woolcock, 2014).

Several researches related to technology indicates the power of the screen (Essig, 2012). While screen technology offers a wide range of facilities such as entertainment, education, socialization, it exposes individuals a lot of patterns, colored texts, animations and exaggerated graphics which are intense stimuli to the eye. It is not possible to consume all these stimuli without developing a mindset based on them. This new way of thinking is screen thinking style, which is moving, shifting, superficial and temporary (Güneş, 2010). Eyes are less cropped and more contracted to provide more concentration when exposed to intense stimuli, differently from its normal function. As a result of this, "computer vision syndrome" may occur (Portello, 2013). It was indicated that humans are sensitive especially to the blue light (short wavelength) emitted from the screens of technological devices (LED); it affects the biological rhythms, sleep and cognitive performance levels (Cajochen et al., 2011) and causes obesity affecting the speed of metabolism and insulin resistance (Cheung et al., 2016). Screen, with these kinds of characteristics, is one of the reasons causing to addiction the device used (Roberts, 2014). It has also been found that screen size may be related to addiction, which supports this argument (Wibirama & Nugroho, 2017).

Excessive screen use was found to be associated with the delays in cognitive, language, academic and psychosocial developments in terms of age; obesity, sleep disorders, differentiation in body image, aggressive and violent behavior along with the decrease in the physical activity; depression, anxiety, loneliness, compulsion, suicidal tendency, attention deficit and hyperactivity along with digital games (Lowry, 2002; Whang et al., 2003; Vandewater, 2004; Yoo et al., 2004; Thompson & Christakis, 2005; Kim et al., 2006; Schmidt, 2009; Tomopoulos et al., 2010; Pagani, 2010; Linebarger & Vaala., 2010; Lemmens, 2011; Madran Demirtaş & Çakılcı Ferligül, 2014; Maras et al., 2015; Mohaç, 2015; Vandenbosch & Eggermont, 2016). In the light of these findings American Academy of Pediatrics (AAP) considered the research related to digital media and digital games as screen use and recommended not to use screen until two years old and then limited and controlled use in terms of age (Reid Chassiakos, 2016). It is, in fact, remarkable to see this information in the literature on various platforms with the aim of raising awareness. Nevertheless, persistence in the use of the screen among children at full speed may make adults think that they do not know how to manage the situation with children or what to replace with screen.

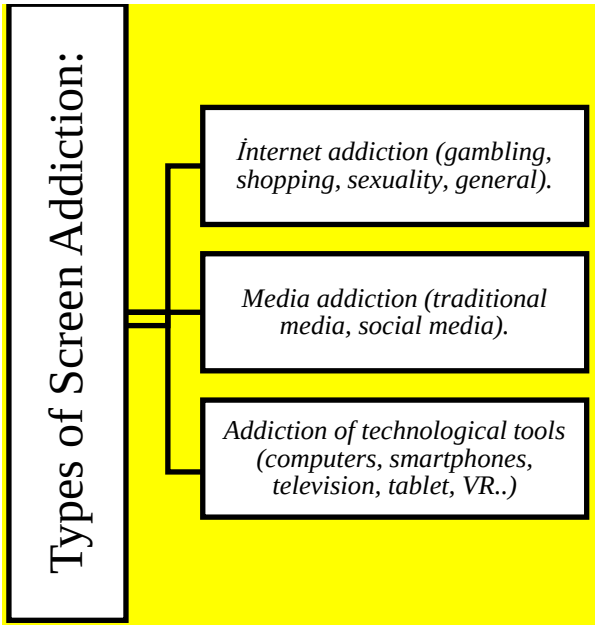
Information Screen Addiction and Subtypes

In this section, types of technology addictions reached via the screen, which the individual establishes a "symbiotic" relationship and perceives it as natural and invisible, are explained.

Theoretical Background

Intensive commucation with information technologies in the modern world improves human capabilities while it causes behavioral addiction with biological, psychological and social aspects at the same time at intensive and long-term use. Although addictions have been known as the use of cigarettes, alcohol and substances; behavioral or operational addictions such as exercise, gambling,

sex, shopping have also been identified (Griffiths, 1996; Demetrovics & Griffiths, 2012). Research shows that when individuals generally repeat behaviors, repetation triggers biochemical processes in the body that have the potential to cause addiction and creates particular effects of reward (Alavi et al., 2012). Young (1998) stated that the basic dynamics of addiction are not the internet itself, but the forms of use provided by the internet. Forms of using screen and Internet together is considered significant for identifying the type of addiction and the treatment (Ögel, 2016). Literature on screen addictions classifies the main addictive dynamics as following: Digital interaction and virtual environment through the screen. Excessive and uncontrolled consumption of both separately or together may cause addiction. In this context, although research are carried out on technology addiction, internet addiction, social media addiction, digital game addiction, computer addiction, smartphone addiction; screen addiction is the subject and includes all of them (Griffiths, 1996; Young, 1998; Shapira et al., 2003; Kuss, 2011; Kwon et al., 2013). Screen addiction subtypes are shown on the Scheme 1**.**



Scheme 1: Types of Screen Addiction:

Internet Addiction

Bill Gates defined internet as "information highway" connecting via screen in 1999 and it is regarded as the "active ingredient" of technology addictions (Savcı & Aysan, 2017). Number of internet users is estimated as 4,156,932,140 all over the world, and 56 million in Turkey (Internet World Stats, 2017). Number of internet users increase in parallel as the days spent on the internet increase. An average internet user spends approximately 6 hour a day online while this is about 7 hours in Turkey (We Are Social, 2018). Long-term and intense use of internet leads to addiction. It was firstly used as internet addiction in the literature but researchers also named Internet addiction as internet dependency, problematic internet use, pathological internet use, excessive internet use, internet abuse, internet addiction disorder, cyberaddiction (Young, 1996; Kaltiala-Heino et al., 2004; Young, 2004; Young & Case, 2004; Vaugeois, 2006; Tvedt, 2007; Weinstein & Lejoyeux, 2010). Different concepts were used because of the different opinions about the cause of problem (Dinçer, 2016a). Internet addiction and diagnostic criteria were firstly defined by Goldberg in 1996 to criticize the DSM system (Şenormancı et al., 2010). Young developed the first measuring tool "Internet Addiction Test" by specifying the criteria for Internet addiction (Kutlu et al., 2016). According to Young, diagnostic criteria for Internet addiction are 1) excessive mental activities (obsessive status, attributing importance), 2) increasing need to use (tolerance), 3) failed attempts to control, reduce or completely quit (relapse), 4) feelings of anxiety, collapse (depression) or anger in case of reduction or complete cessation (withdrawal), 5) being online on the internet more than the planned time (uncontrolled use), 6) having problems with family, school, work and friends due to excessive use of the internet, jeopardising or losing an opportunity of education or career (abuse, problem), 7) lying others about the amount of time spent on the internet (conflict), 8) using internet to escape from the problems or get away from the negative feelings (changing feelings). Having at least five of the eight criteria within 6 months is regarded as Internet addiction (Young, 1998). Based on these criteria Davis divided Internet addiction into two subgroups as General Pathological Internet Use (GPIU) and Specific Pathological Internet Use (SPIU). While GPIU is excessive use without a specific purpose, SPIU excessive interne use for a specific purpose such as gambling, sex, internet shopping (Dinç, 2016b).

Young (2000) also classified five general subtypes of Internet addiction according to most frequent online types. These are 1) Cyber-sex addiction, 2) Cyber-relationship addiction, 3) Net compulsions (virtual gambling, shopping or transactions), 4) Information overload (compulsive internet surfing or database searches) and 5) Computer addiction.

Digital interaction, ultimately, resulting from an unnatural communication with the Internet in terms of the receiver and channel of the information may cause addiction on individuals. Lack of communication skills and poor quality of communication in real life increases the need for communication even further, and the individual uses the opportunities provided by the Internet in a problematic way to meet this need of communication and establish meaningful relationships. The individual who establishes this poor-quality communication, experiences the "internet paradox" (Kraut, Kiesler, Boneva, Cummings, Helgson & Crawford, 2002). Similarly, several research about the internet indicated that the conditions which cause excessive and uncontrolled internet use is caused by lack of social support and inadequate communication skills (Mesch, 2001; Ceyhan, 2011; Savcı & Aysan, 2017). In some studies, individuals were found to have no lack of communication skills, on the contrary, individuals use the internet to increase social support in real life and use their communication skills to communicate with more people (Savci & Aysan, 2016). However, people may experience the internet paradox due to the unnatural and poor-quality structure of digital interaction.

Media Addiction

Considering that internet is mostly used for social media, social media addiction comes first in SPIU. Including television media which has limited interaction, beside "active ingredient" internet, as "digital media", "electronical media" and "screen media", research exploring abuse and addiction of media showed that it is quite important especially for youth in the context of identity and intimacy development and they are mostly affected group by the harmful effects (Baer et al., 2012; Michikyan & Suarez-Orozco, 2016; Reid Chassiakos, 2016; Domoff et al. 2017; Görmez & Örengül, 2017). It was reported that excessive TV watching causes delay in cognitive, academic, psychosocial and language development, obesity with decrease in physical activity, being exposed to cyber bullying according to used social media types and using habits and change in body image due to appearance oriented media (Lowry, 2002; Vandewater, 2004; Schmidt, 2009; Tomopoulos et al., 2010; Linebarger & Vaala, 2010; Pagani, 2010; Vandenbosch & Eggermont, 2016; Aslan et al., 2017). In the light of these findings, American Academy of Pediatrics suggested families to limit the digital media in terms of age groups (Reid Chassiakos, 2016).

The concept of "Fear of Missing Out" explains that digital interaction gradually increases the need for communication and shows signs of deprivation as individuals constantly having anxiety to learn what others are doing as a result of being rewarded with interaction in social networks (Gökler et al., 2015; Przybylski et al, 2013; Hato, 2013). In addition, while media addiction theory reveals the need of society and the individual for mass communication tools; it reveals perception, emotion and behaviour changes and addiction formation of individual on interaction basis, whether it is active as in social media and online games or passive as in television. According to the media addiction theory, people living in masses need information to make any kind of decisions, they establish social relations with the people whom they acquire information, however, people who have difficulty in face-to-face communication and building social relationships due to class differences in social structures and personal differences, get addicted on the media to find a type of social role that they can use to build social relationships and to get information they can use to make decisions (Işık, 2009).

Digital Game Addiction

Digital games, which are fun, independent from the real world and used via screen, are another purpose of consuming technology (Garris R. et al., 2002). Digital games can be played online or offline via technological tools such as computer, console, smart phones or virtual reality and cause addictions in problematic using situations (Griffiths & Davies 2005; Young, 2009; Irmak Yalçın A. & Erdoğan S., 2016). American Psychiatric Association included digital game addiction as Internet Gaming Disorder in the third research appendix of Diagnostic and Statistical Manual in 2013. Digital game addiction was found to be associated with aggression, obesity, loneliness, depression, suicidal tendencies, compulsion, attention deficit and hyperactivity (Whang et al., 2003; Yoo et al., 2004; Kim et al., 2006; Lemmens, 2011; Madran Demirtaş & Çakılcı Ferligül, 2014; Mohaç, 2015).

According to Yee (2007), there are three components that cause game addiction. These are

"success", "social compliance" and "diving into game". The desire to compete with other players in the success component and the desire for socialization, connecting relationships and taking part in teamwork in the social component may occur as a result of digital interaction and can be damaging for real life. Diving into play component is related to the virtual environment. Virtual environment is intriguing, unlimited, free, artificial and anonymous. With all these features, it is open to being explored, idealized, personalized and allows to get away from the facts. For these reasons, it has a supportive structure for the addiction. Recently, human beings have begun to isolate themselves from reality and started to attribute special meanings to the virtual world in every opportunity with the development of mobile technological tools (Karadağ, 2016).

Online and offline digital games give individuals profiles to interact, manage and express themselves regularly. Considering the connection with digital games, these digital profiles form a virtual self (Gosling et al., 2011). Profiles created in virtual environment can be formed according to idealized self instead of real self. Virtual environment allows the ideal self to be actualized in an easy way (Monago et al., 2008). In this case, the virtual self can take over the real identity and affect thoughts, emotions and personality not only online but also offline. On the other hand, there are also studies showing that the virtual self is not idealized but only an exaggerated expression of the real self (Back et al., 2010).

Moreover, studies on virtual bullying, which is frequently seen in digital games, showed that lack of empathy and emotion is one of the reasons for bullying (Topçu, 2008; İnselöz-Türkileri Uçanok, 2013). Feelings are shallow and empathy is limited in the unlimited and free world of virtual environment. Therefore, studies indicating that virtual environment leads individuals to violence is remarkable (Madran Demirtaş & Çakılcı Ferligül, 2014).

Conclusion

This study aimed to determine the subtypes of screen addiction and classify it finding out the underlying reasons. Dynamics and underlying reasons of screen addiction classified as Internet addiction (gambling, shopping, sex, general), media addiction (traditional media, social media), digital gaming addiction (online, offline, single-player, multiplayer, depending on the type), technological tools addiction (computers, smart phones, television, tablet, VR, etc.) are found to be important for the explanations of addiction and the diagnosis and treatment of individuals with addiction.

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